January 1989

TRINITROTOLUENE

Health Advisory

Office of Drinking Water
U.S. Environmental Protection Agency
Washington, DC 20460

Health Advisory on 2,4,6-Trinitrotoluene

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January 1989

PREFACE

This report was prepared in accordance with the Memorandum of Understanding between the Department of the Army, Deputy for Environment Safety and Occupational Health (OASA(I&L)), and the U.S. Environmental Protection Agency (EPA), Office of Drinking Water (ODW), Criteria and Standards Division, for the purpose of developing drinking water Health Advisories (HAs) for selected environmental contaminants, as requested by the Army.

Health Advisories provide specific advice on the levels of contaminants in drinking water at which adverse health effects would not be anticipated and which include a margin of safety so as to protect the most sensitive members of the population at risk. A Health Advisory provides health effects guidelines, analytical methods and recommends treatment techniques on a case-by-case basis. These advisories are normally prepared for One-day, 10-day, Longer-term and Lifetime exposure periods where available toxicological data permit. These advisories do not condone the presence of contaminants in drinking water; nor are they legally enforceable standards. They are not issued as official regulations and they may or may not lead to the issuance of national standards or Maximum Contaminant Levels (MCLs).

This report is the product of the foregoing process. Available toxicological data, as provided by the Army, on the munitions chemical 2,4,6-trinitrotoluene (TNT) have been reviewed and relevant findings are presented in this report in a manner so as to allow for an evaluation of the data without continued reference to the primary documents. This report has been submitted to critical internal and external review by the EPA.

A companion document, "Data Deficiencies/Problem Areas and Recommendations for Additional Data Base Development for Trinitrotoluene" is included in this report.

I would like to thank the authors, Ms. Loretta Gordon and Dr. William Hartley, who provided the extensive technical skills required for the preparation of this report. I am grateful to the members of the EPA Tox-Review Panel who took time to review this report and to provide their invaluable input, and I would like to thank Dr. Edward Ohanian, Chief, Health Effects Branch, and Dr. Joseph Cotruvo, Director, Criteria and Standards Division, for providing me with the opportunity and encouragement to be a part of this project.

The preparation of this Advisory was funded in part by Interagency Agreement (IAG) 85-PP5869 between the U.S. EPA and the U.S. Army Medical Research and Development Command (USAMRDC). This IAG was conducted with the technical support of the U.S. Army Biomedical Research and Development Laboratory (USABRDL).

Krishan Khanna, Project Officer
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I. INTRODUCTION

The Health Advisory (HA) Program, sponsored by the Office of Drinking Water (ODW), provides information on the health effects, analytical methodology and treatment technology that would be useful in dealing with the contamination of drinking water. Health Advisories describe nonregulatory concentrations of drinking water contaminants at which adverse health effects would not be anticipated to occur over specific exposure durations. Health Advisories contain a margin of safety to protect sensitive members of the population.

Health Advisories serve as informal technical guidance to assist Federal, State and local officials responsible for protecting public health when emergency spills or contamination situations occur. They are not to be construed as legally enforceable Federal standards. The Advisories are subject to change as new information becomes available.

Health Advisories are developed for One-day, Ten-day, Longer-term (approximately 7 years, or 10% of an individual's lifetime) and Lifetime exposures based on data describing noncarcinogenic end points of toxicity. Health Advisories do not quantitatively incorporate any potential carcinogenic risk from such exposure. For those substances that are known or probable human carcinogens, according to the Agency classification scheme (Group A or B), Lifetime HAs are not recommended. The chemical concentration values for Group A or B carcinogens are correlated with carcinogenic risk estimates by employing a cancer potency (unit risk) value together with assumptions for lifetime exposure and the consumption of drinking water. The cancer unit risk is usually derived from the linear multistage model with 95% upper confidence limits. This provides a low-dose estimate of cancer risk to humans that is considered unlikely to pose a carcinogenic risk in excess of the stated values. Excess cancer risk estimates may also be calculated using the One-hit, Weibull, Logit and Probit models. There is no current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than another. Because each model is based upon differing assumptions, the estimates that are derived can differ by several orders of magnitude.

II. GENERAL INFORMATION AND PROPERTIES

Trinitrotoluene (TNT) or, more specifically, α-TNT is the common designation for 2,4,6-trinitrotoluene, the most widely used military high-explosive (Castorina, 1980). For purposes of this HA, the synonym, TNT, will be used throughout to refer to 2,4,6-trinitrotoluene. Along with TNT, the symmetrical isomer, five meta or unsymmetrical trinitrotoluene isomers are found in the crude product resulting from the nitration of toluene with nitric acid in the presence of sulfuric acid. The nitration occurs in a step-wise fashion by a batch or continuous process.

The continuous process as employed at the Radford Army Ammunition Plant (RAAP), a prototype for Army Ammunition Plants (AAPs), utilizes 99% nitric acid and 44% oleum (109% sulfuric acid, a solution of sulfur trioxide in anhydrous sulfuric acid; Small and Rosenblatt, 1974) to nitrate toluene in six stages to crude TNT which is then subjected to purification with aqueous sodium sulfite (sellite) (Ryon et al., 1984). This process has been further modified to employ eight nitrator vessels fitted with dynamic (centrifugal) separators, thereby ensuring a greater degree of safety and efficiency. The purification process consists of two acid washes, three sellite washes and two post-sellite washes.

The crude TNT contains approximately 5% of the meta-isomers. These are reduced to about 0.6% by the sellite purification. Crude TNT also contains approximately 1% of the six dinitrotoluene (DNT) isomers, which are not removed during purification, and slightly more than 1% oxidation products, which are reduced to <1% by purification. Three additional impurities, amounting to <1%, are introduced by the sellite process (Ryon et al., 1984). Total impurities constitute not more than 3.24% of the finished TNT (Pal and Ryon, 1986).

The resulting monoclinic rhombohedric crystals, as described in Rosenblatt et al. (1971), when very pure, melt at 80.99°C, although a melting point as high as 81.6°C has been reported and 80.65°C is a commonly accepted figure (80.1 - 81.6°C). The color is usually pale yellow, but a chromatographically purified sample has been described as faintly yellow to pure white. A boiling point of 210° to 212°C at 10 to 12 mm Hg has been determined. The specific gravity has been variously reported over the range of 1.3 to 1.6 gm/cc. Although the solubility of TNT in water at 20°C is only 0.013% (130 mg/L), this is significant for pollution and health issues. Its solubility in organic solvents runs much higher, e.g., 109 gm/100 g of acetone at 20°C.

Two grades of TNT are used for military purposes and their purities are measured by the solidification point (also termed freezing point or setting point), which is considered more reproducible than a melting point. Grade III, the more highly purified grade, has a solidification point of 80.4°C, minimum, and exists as a fine crystalline form (Department of the Army, 1967).

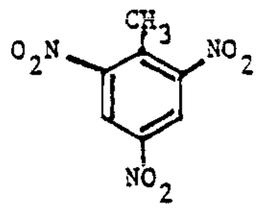
General chemical and physical characteristics of TNT are presented in Table II-1.

Trinitrotoluene is among the least impact- and friction-sensitive of the high explosives and the impurities formed during its production (except for tetranitromethane) do not affect its sensitivity. It can be further desensitized, however, by adding certain stabilizing substances in small quantity (1% to 2%) (Rosenblatt et al., 1971).

The chemical stability of TNT is such that, even at 150°C, it undergoes no great decomposition in 40 hours. Molten TNT can be stored at 85°C for 2 years without any decrease in purity. TNT has been found to withstand storage at magazine temperatures for 20 years without any measurable deterioration. Furthermore, moisture has no effect on the stability of TNT, which is unaffected by immersion in sea water (Department of the Army, 1967).

TABLE II-1

GENERAL CHEMICAL AND PHYSICAL PROPERTIES OF 2,4,6-TRINITROTOLUENE[a]

CAS Number	118-96-7
Names	TNT, α-trinitrotoluol, 1-methyl-2,4,6-trinitrobenzene, trotyl, tolite, triton, tritol, trilite, α-TNT
Molecular weight	227.13
Empirical formula	$C_7H_5N_3O_6$
Structure	
Color	Yellow to white
Physical state	Monoclinic rhombohedral crystals
Specific gravity	1.654
Liquid density	1.465 g/cm^3
Vapor pressure	0.053 mm (85°C); 0.106 mm (100°C)
Solubility characteristics	Water: 0.013 g/100 g (20°C) Carbon tetrachloride: 0.65 g/100 g (20°C) Toluene: 55 g/100 g (20°C) Acetone: 109 g/100 g (20°C)
Melting point	80.1 - 81.6°C
Boiling point	210°C (10 mm) - 212°C (12 mm)
Freezing point	80.75 ± 0.05°C
Flash point	240°C (explodes)
Conversion factor	1 ppm = 9.28 mg/m^3 (25°C; 760 mmHg) 1 mg/m^3 = 0.108 ppm (25°C; 760 mmHg)

[a] References: Clayton and Clayton (1981); Rosenblatt et al. (1973); Department of the Army (1967); Windholz (1976); Zakhari and Villaume (1978)

III. OCCURRENCE

Trinitrotoluene was produced and used on an enormous scale during World War I and World War II and may be considered the most important military bursting charge explosive. It has found wide application in shells, bombs, grenades, demolition explosives and propellant compositions (Department of the Army, 1967).

Trinitrotoluene is manufactured primarily by the continuous process, as described above, in Army Ammunition Plants (AAPs). Production from 1969-1971 was reported as 45 million pounds/month with a capacity of 85 million pounds/month (Ryon et al., 1984). It has been reported that as much as one half million gallons of wastewater have been generated per day by a single plant involved in the production of TNT (Hartley et al., 1981).

Trinitrotoluene wastes have a unique terminology as described in Rosenblatt et al. (1973). "Nitrobodies" include TNT, other TNT isomers, products from the sellite purification process and by-products from the production process. The spent sellite washings are high in solids content and are called "red water". Ryon et al. (1984) have reported that "TNT is the largest single non-polar component". The major organic components identified are 2,4-dinitrotoluene-3-sulfonate and 2,4-dinitrotoluene-5-sulfonate, which make up approximately one-third of the polar organic fraction. Such water is intensely red-colored and either is sold to paper mills for sulfur content or is concentrated by evaporation and incinerated. It is not amenable to purification and, because it is classified by EPA as a hazardous waste, it cannot be discharged into streams.

"Pink water" comes from both manufacturing plants and from load, assemble and pack (LAP) facilities. That from manufacturing plants can arise from Mahon fog filter effluents and nitrator fume scrubber discharges and is known to consist of the DNTs. While not positively identified, these two sources of "pink water" are also believed to contain all TNT isomers, mononitrotoluenes (MNTs) and possibly dinitro-m-cresols arising from the displacement of a nitro group on TNT isomers. Additionally, "pink water" from manufacturing plants arises from "red water" distillates (evaporator condensate from concentration process) and consists of DNTs, while those from finishing building hood scrubber and wash-down effluents are also believed to contain primarily DNTs. Spent acid recovery wastes may be an additional source of "pink water" generated during the manufacturing process (Dacre and Rosenblatt, 1974). On the other hand, "pink water" from LAP facilities, resulting primarily from shell washout operations, contains essentially pure TNT, usually contaminated with hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) or other additives. The pink color — pale straw to brick red — arises under neutral or basic conditions, especially when the wastes are exposed to sunlight (Rosenblatt et al., 1973).

A number of photodegradation products of TNT have been identified in organic solvent extracts of "pink water". Those degradation products that are water soluble (but not extractable by organic solvents) have not been fully characterized; however, as many as thirty components of condensate wastewater (i.e. steam distillates arising from the concentration of "red water" by evaporation) obtained from the Volunteer AAP have been identified and quantified (Table III-1). Other constituents not derived from TNT degradation include the toxicologically significant DNT isomers, particularly 2,4- and 2,6-DNT (Dacre and Rosenblatt, 1974).

Table III-1. 90th Percentile Concentrations and Relative Concentrations Determined for Condensate Components[a]

Condensate Component	90th Percentile Concentration[b] (mg/liter)	Concentration[c] (%)
Toluene	0.200	0.590
2-Nitrotoluene (NT)	0.030	0.089
4-Nitrotoluene	0.100	0.295
3-Nitrobenzonitrile[d]	0.013	0.035
4-Nitrobenzonitrile[d]	0.009	0.027
2-Amino-4-NT	0.033	0.097
2-Amino-6-NT[d]	0.010	0.030
3-Amino-4-NT[d]	0.027	0.080
3-Methyl-2-nitrophenol	0.012	0.035
5-Methyl-2-nitrophenol	0.032	0.094
1,3-Dinitrobenzene (DNB)	4.000	11.803
2,3-Dinitrotoluene (DNT)	0.400	1.180
2,4-DNT	14.700	43.377
2,5-DNT	0.400	1.180
2,6-DNT	7.300	21.541
3,4-DNT	0.500	1.475
3,5-DNT	0.520	1.534
3,5-Dinitroaniline[d]	0.058	0.171
1,5-Dimethyl-2,4-DNB (DNX)	0.390	1.151
2-Amino-3,6-DNT	0.030	0.089
2-Amino-4,6-DNT	0.020	0.059
3-Amino-2,4-DNT	1.500	4.426
3-Amino-2,6-DNT	1.200	3.541
4-Amino-2,6-DNT	0.600	1.770
4-Amino-3,5-DNT	0.200	0.590
5-Amino-2,4-DNT	0.700	2.066
2,4-Dinitro-5-methylphenol[d]	0.085	0.251
1,3,5-Trinitrobenzene (TNB)[d]	0.153	0.451
2,3,6-Trinitrotoluene (TNT)[d]	0.268	0.791
2,4,6-TNT	0.400	1.180

a/ Reference: Spanggord et al., 1978.

b/ Determined by cluster analysis of data points from two studies via a specially developed computer program.

c/ The 90th percentile concentration of those compounds appearing in at least 10% of the samples/sum of the concentrations of all components.

d/ Compounds were not present in 10% of the samples. Value given represents the mean of the non-zero values.

IV. ENVIRONMENTAL FATE

Several studies have been conducted to determine the environmental fate of TNT in wastewater. Based on a review of the available literature and the physical and chemical properties of TNT, Spanggord et al. (1980a) indicated that sediment adsorption and volatilization were not likely to be significant fate processes, but further investigation was recommended. Degradation via photolysis and biotransformation, but not hydrolysis, were considered significant.

Based on the weight of evidence in experiments conducted under a wide range of conditions, Tucker et al. (1985) reported that adsorption in soil would be an important process affecting the migration of TNT, with the cation exchange capacity and organic carbon content most critical in determining the degree of adsorption. Molecular diffusion was also considered an important factor and was related largely to percolation rate while vapor phase diffusion would only be significant in arid climates where soil clay content was low.

In contrast, Sikka et al. (1980) showed that sorption of TNT to pond sediment is measurable but not extensive, is correlated to time and concentration and is stongly, perhaps irreversibly, bound. Sorption of TNT to sediment is also directly related to pH and temperature. The degradation products of TNT also appear to be adsorbed to sediment.

The photolytic degradation of TNT wastewater is well known, as indicated by the formation of "pink water", and numerous degradation products of TNT have been identified. Laboratory studies have determined that photolysis is the primary process involved in the loss of TNT from the environment, and that the concentration of TNT in wastewater will decline rapidly within a short distance of its discharge point. Furthermore, photolysis is accelerated by the products formed during the photolytic process as well as by natural substances. Using a computer model, Spanggord et al. (1980b) estimated probable concentrations of TNT and its degradation products in water bodies. Based on detailed kinetic investigations, an environmental half-life of 3 to 22 hours was calculated. Burlison (1980) determined that the major transformation product in natural water was 1,3,5-trinitrobenzene.

In contrast, Spanggord et al. (1980b) demonstrated a slow biotransformation of TNT in natural water, even in the presence of small quantities of organic nutrients. The major process of transformation was reported to be via nitro-group reduction with no ring cleavage detected. The 2-amino and 4-aminodinitrotoluenes were the major biotransformation products (Burlison, 1980). Half-lives of 8 to 25 days were experimentally estimated for TNT.

Similarly, Spanggord et al. (1981) determined that volatilization of TNT was very slow and that actual rates were consistently lower than those estimated by physical and chemical properties.

V. PHARMACOKINETICS

Trinitrotoluene is absorbed by inhalation, ingestion or skin contact, rapidly biotransformed in the liver, excreted in the urine and distributed to the organs; however, rapid clearance precludes extensive bioaccumulation. It is metabolized primarily by reduction of the nitro group and, to a lesser extent, by oxidation of the methyl group and ring hydroxylation. Glucuronide conjugates have been found and 4-amino-2,6-dinitrotoluene is the main metabolite identified (Ryon, et al., 1984). Available data indicate that TNT is well absorbed (more than 50% of the administered dose in most experiments) in a variety of test species. The major route of excretion is via urine with some (generally the balance of the recovered dose) in the feces and gastrointestinal (GI) tract plus its contents. Distribution to other tissues is usually less than 1%. Only negligible amounts (0.1%) were recovered in expired air. Several metabolites have been identified in the urine of various species including hydroxylated, mono- and dinitro and mono- and diamino derivatives.

A. Absorption

Lee et al. (1975) studied the absorption of TNT in Charles River CD female rats (175 to 250 g) that were fasted overnight before being given a single oral dose of approximately one tenth of the LD_{50} of the test compound (i.e., 82 mg/kg), spiked with 10 μCi of the ^{14}C-labelled compound. The test material was suspended in peanut oil and administered via intragastric intubation at a volume of 1 ml/100 g body weight. After dosing, each rat was placed in a "Roth-Delmar" metabolism cage where feces and urine were collected separately. At the termination of each experiment, each rat was anesthetized, and aortic blood was collected in a heparinized syringe. Liver, kidneys, brain, lung, thigh muscle, GI tract plus contents and the feces were homogenized and assayed for radioactivity at 24 hours after TNT administration.

Approximately 60% to 74% of the administered dose was absorbed in the 24-hour period. Most of the absorbed radioactivity was excreted in the urine, averaging 53.3% of the administered dose, with about 26% of the radioactivity found in the GI tract and the feces. A negligible amount of radioactivity was recovered in the expired air.

From the same laboratories, Hodgson et al. (1977) and El-hawari et al. (1981) reported on the absorption of TNT administered as a single oral dose at 100 mg/kg (spiked with 25 μCi/kg of the ^{14}C-TNT) to male and female Charles River CD or Sprague-Dawley rats (200 to 300 g) using the same procedure as described by Lee et al. (1975). Table V-1 compares the results of these three studies. About 53% to 65% of the administered radioactivity appeared in the urine within 24 hours. Most of the remaining radioactivity was recovered in the GI tract plus contents and in the feces, averaging 26% to 38% of the dose. The urine was bright red in color indicating the presence of metabolic products.

Table V-1
Percentage of Orally Administered TNT[a/]
Recovered in Rats in 24 Hours

Strain Dose	Charles River CD 82 mg/kg	Charles River CD 100 mg/kg		Sprague-Dawley CD 100 mg/kg	
	Female(3)[b/]	Male(4)	Female(4)	Male(4)	Female(4)
Total Recovery	82.1	91.3	102.0	91.6	102.4
Feces	5.5	8.1	2.1	8.0	2.0[c/]
GI Tract plus contents	20.7	29.7	33.9	29.8	33.9
Urine	53.3	52.7	64.5	52.7	64.5[c/]
Expired Air	0.1	NA[d/]	NA	NA	NA
Other Tissues	2.5	0.8	1.5	1.1	2.0
Author	Lee et al. (1975)	Hodgson et al. (1977)		El-hawari et al. (1981)	

a/ ring-UL-^{14}C; standard deviations omitted.
b/ (n)=numbers of animals analyzed.
c/ Significantly different ($p<0.05$) from males.
d/ Not analyzed.

Similar studies were conducted by these same authors (Hodgson et al. 1977; El-hawari et al., 1981) in albino CD1 mice, New Zealand rabbits and beagle dogs. Results are compared in Tables V-2, V-3 and V-4, respectively.

As seen in Table V-2, about 42% to 60% of the radioactivity administered to mice appeared in the urine within 24 hours. A range of 16% to 55% of the dose remained in the GI tract plus contents and the feces. As in the rat, mouse urine was bright red.

In rabbits, Table V-3, about 66% to 79% of the administered radioactivity appeared in the urine within 24 hours. The radioactivity in the GI tract plus contents and in the feces averaged 6% to 13% of the dose. Unlike that of the rat and mouse, rabbit urine did not contain a red pigment.

The results for dogs were similar to those for rabbits. As indicated in Table V-4, about 56% to 60% of the administered radioactivity appeared in the urine within 24 hours. The GI tract plus contents and the feces contained 15% to 21% of the dose. Similar to the rabbit, the urine did not contain a red pigment.

El-hawari et al. (1981) also compared the 24-hour recovery of radiolabelled TNT in all four species following a single oral dose of 50 mg/kg to each. As seen in Table V-5, rats, mice and dogs excreted relatively equivalent amounts in the urine whereas, in rabbits, the urinary excretion of radioactivity was higher with a proportional decrease in the percent of radioactivity recovered from the GI tract plus contents and feces. Even at this 10-fold higher dose, red pigment was not detected in the urine of the rabbit and dog. This red pigment has been reported to occur in the urine of humans "poisoned" with TNT (Hassman, 1972 as cited in El-hawari et al., 1981).

El-hawari et al. (1981) studied species differences in the absorption and excretion of TNT as a function of the route of administration. Swiss albino mice (20 to 30 g), Sprague-Dawley rats (200 to 300 g), New Zealand rabbits (3 to 4 kg), and beagle dogs (8 to 14 kg) were used in parallel oral and dermal studies.

In the oral studies, test animals were fasted overnight before receiving single doses of ^{14}C-TNT dissolved in peanut oil. In the dermal study, the fur on the back of the test animals was removed with electric clippers and, on the following day, the ^{14}C-TNT in peanut oil was spread over the depilated areas.

Rats and mice were dosed at 50 mg/kg and dogs and rabbits were dosed at either 5 or 50 mg/kg. Animals were placed in individual metabolism cages for the separate collection of urine and feces. At 24 hours after dosing, animals were anesthetized and killed, and blood, liver, kidneys, lungs, spleen, brain, skeletal muscle, fat (retroperitoneal), and the GI tract plus contents were analyzed for radioactivity.

Table V-2

Percentage of Orally Administered TNT[a/] (100 mg/kg) Recovered in Albino CD1 Mice in 24 Hours

	Male (7)[b/]	Female (8)	Male (7)	Female (8)
Total Recovery	101.4	86.8	80.0	60.4[c/]
Feces	42.6	18.2	22.0	9.0[c/]
GI Tract plus contents	12.4	7.4	13.4	7.4[c/]
Urine	44.7	60.5	41.9	42.9
Other Tissues	1.7	0.7	2.7	1.1
Author	Hodgson et al. (1977)		El-hawari et al. (1981)	

a/ ring-UL-^{14}C; standard deviations omitted.
b/ (n)=numbers of animals analyzed.
c/ Significantly different ($p<0.05$) from males.

Table V-3

Percentage of Orally Administered TNT[a/] (5 mg/kg) Recovered in Rabbits in 24 hours

	Male (3)[b/]	Female (3)	Male (3)	Female (3)
Total Recovery	78.1	94.7	77.6	88.9
Feces	1.8	1.8	1.8	1.8
GI Tract plus contents	7.5	10.8	7.5	4.7
Urine	66.3	78.9	66.3	78.8
Other Tissues	2.5	3.2	2.0	3.6
Author	Hodgson et al. (1977)		El-hawari et al. (1981)	

a/ ring-UL-^{14}C; standard deviations omitted.
b/ (n)=numbers of animals analyzed.

Table V-4

Percentage of Orally Administered TNT[a/] (5 mg/kg)
Recovered in Dogs in 24 hours

	Male (3)[b/]	Female (3)	Male (3)	Female (3)
Total Recovery	79.6	87.5	77.4	88.2
Feces	5.4	16.7	5.4	16.8[c]
GI Tract plus contents	9.9	4.3	10.0	4.4
Urine	59.1	60.0	55.9	60.2
Other Tissues	5.2	6.5	6.1	6.8
Author	Hodgson et al. (1977)		El-hawari et al. (1981)	

a/ ring-UL-^{14}C; standard deviations omitted.
b/ (n)=numbers of animals analyzed
c/ Significantly different ($p<0.05$) from males

Table V-5

Percentage of Orally Administered INT[a/] (50 mg/kg)
Recovered in 24 hours

	Rats (3)[b/]	Mice (8)	Rabbits (2)	Dog (1)
Total Recovery	92.2(81.3)[c/]	94.4	103.6	94.2
Feces	10.7 (2.1)	24.1	5.1	22.2
GI Tract plus contents	20.2(35.3)	10.2	22.7	1.7
Urine	59.5(42.5)[d/]	59.0[d/]	74.3	61.0
Other Tissues	1.8 (1.4)	1.1	1.5	9.3
Author	El-hawari et al. (1981)			

a/ ring-UL-^{14}C; specific activity 19.76 µCi/mg; single dose; standard deviations omitted.
b/ (n)=numbers of animals analyzed.
c/ Male(Female) values.
d/ Bright red pigment in urine.

Absorption of TNT was confirmed by both the oral and dermal routes of administration. The authors reported that TNT is readily absorbed after oral administration with rabbits and dogs appearing to absorb more TNT than rats and mice. The extent of oral absorption is reported as approximate based on the recovery data since the extent of biliary excretion and enterohepatic circulation was not evaluated. The dermal experiments confirmed the potential absorption of TNT through the skin with absorption being highest in male rabbits (57-68%) followed by male mice (42%), female rats (25%), male rats (23%), and male dogs (16-17%). In all species, total elimination of the administered radioactivity was reported to be lower after dermal application as compared to oral administration. Table V-6 compares the total recovery as a percent of the administered dose in rats, mice, rabbits and dogs by both routes. As in the previously described oral studies, red pigment was present in the urine of rats and mice treated dermally with a single application of ^{14}C-TNT but not in the urine of dogs and rabbits.

In an effort to simulate inhalation exposure, El-hawari et al. (1981) instilled 50 mg/kg of TNT suspended in methyl cellulose into the trachea of anesthetized, tracheotomized, male and female Sprague-Dawley rats. Concurrent experiments were performed in rats treated orally with the same dose of ^{14}C-TNT under the same experimental conditions. After 4 hours, both groups of rats were sacrificed and tissues and bladder urine were collected for analysis of radioactivity.

After intratracheal instillations, the authors reported that the rate of absorption was faster, and the extent of absorption greater and more uniform with less individual variation as compared to orally treated rats. Urinary excretion in intact male rats, 4 hours after intratracheal instillation, averaged 19.3% while, 4 hours after oral administrations, the urinary excretion averaged 14.6%. Urinary excretion in female rats treated intratracheally or orally was somewhat lower, averaging 13.2 and 10.0%, respectively. The authors described the pharmacokinetic behavior of the TNT after intratracheal instillation as "comparable to the behavior usually observed after intravenous administration of other xenobiotics." Direct comparison between intratracheal and dermal routes was not studied. As in the previous studies, the urine of rats treated with a single dose of ^{14}C-TNT by intratracheal instillation contained the characteristic red pigment.

Since the GI tracts of the rats treated by intratracheal instillation contained considerable amounts of radioactivity, some of the rats in this experiment were bile-duct cannulated for collection of bile. Table V-7 compares the recovery of the radioactivity in cannulated and non-cannulated rats four hours after oral or intratracheal treatment with ^{14}C-TNT. Excretion of radioactivity in the urine and bile was higher after intratracheal administration but lower in the GI tract when compared to oral administration. The authors reported that enterohepatic recycling of TNT and/or its metabolites was suggested by a higher recovery of radioactivity in the urine

Table V-6

Total Recovery of ^{14}C-TNT After Oral and Dermal Administration in Various Species[a/]

Species	Sex	Dose mg/kg	Percent of Dose Recovered Oral	Dermal
Rat (3/6)[b/]	Male	50	92.2	22.8[c/]
Rat (3/6)	Female	50	81.3	24.8[c/]
Mice (8/6)	Male	50	94.4	41.7[c/]
Rabbit (3/4)	Male	5	95.6	68.3[c/]
(2/2)		50	103.6	56.9[d/]
Dog (3/3)	Male	5	99.4	16.8[c/]
(1/1)		50	94.2	15.9[d/]

Based on El-hawari et al. (1981)

a/ Standard deviations omitted; fat and skin (including site of application) are not included in recovery estimates.
b/ (n/n) = number of animals evaluated oral/dermal.
c/ Significantly different ($p<0.05$) from oral treatment.
d/ Not statistically analyzed.

Table V-7

Percentage[a] of Orally and Intratracheally Administered ^{14}C-TNT (50 mg/kg) Recovered in Sprague-Dawley Rats in 4 Hours

	Intact		Bile Duct-Cannulated	
	Oral (5)[b]	Intratracheal (6)	Oral (3)	Intratracheal (4)
Total Recovery	95.6	42.9[c]	93.4	46.0[c]
Urine	12.3	16.3	9.6	15.1[c]
GI Tract	76.4	15.2[c]	66.2	2.4[c]
Bile	--	--	10.6	17.1[c]
Other Tissues	6.9	11.4	7.0	11.4

Based on El-hawari et al. (1981)

[a] Average recovery male and female; standard deviations omitted; fat not included in recovery estimates.
[b] (n) = number of animals analyzed.
[c] Significantly different ($p<0.05$) from oral treatment (males and females analyzed separately).

of the intact rats and a radioactivity level in the bile of rats equal to or greater than that excreted in the urine.

B. Distribution

Studies on the recovery of radioactivity from various tissues 24 hours after treatment indicate that retention of ^{14}C-TNT in the tissues of rats, mice, rabbits and dogs is not extensive but that differences between species and routes of administration did occur.

Lee et al. (1975) reported on the distribution of radioactivity from ^{14}C-labelled TNT in Charles River CD female rats. At 24 hours after dosing, small but significant amounts (0.2 to 1.0%) of radioactivity were found in the blood, liver, kidney, and skeletal muscle. Small amounts (<0.1%) were also found in the lungs and brain. The tissue to plasma radioactivity ratio suggested some retention of radioactivity in the liver and kidney.

In the same study, another group of rats received a lethal dose of TNT in order to determine how much of this compound was distributed to the brain. In these animals, only 0.1% of the administered dose was found in the brain at 30 minutes. Small amounts (0.1% to 0.3% of the total administered dose) were also found in the liver, urine, kidneys, and whole blood of these animals.

Data on the distribution of ^{14}C-TNT in four animal species in the study by Hodgson et al. (1977) indicate that there were no major species differences in the distribution of radioactivity in the tissues analyzed. In mice and rats, small amounts (0.2 to 0.7%) of radioactivity were found in the blood, liver and kidneys. The other tissues contained only negligible amounts of ^{14}C. In rabbits, small amounts (0.3 to 1.0%) of radioactivity were found in the blood and liver, while in dogs small but significant amounts of radioactivity were found in the blood, liver and muscle (1.2 to 2.2%). The other tissues contained only negligible ($\leq$ 0.2%) amounts of ^{14}C.

In the study by El-hawari et al. (1981), at 24 hours, blood and tissue of dogs contained a higher percentage of administered radioactivity than did the blood and tissue of rats, mice, or rabbits. Table V-8 compares the radioactivity recovered from various organs, as a percentage of administered dose, in all four species 24 hours after the oral administration of a single 50 mg/kg dose of ^{14}C-TNT. Administration of different doses (100 mg/kg in rats and mice or 5 mg/kg in dogs and rabbits) produced generally comparable tissue levels 24 hours after oral administration. Recovery of ^{14}C-TNT was greatest in liver, skeletal muscle and blood.

Table V-9 compares the tissue-to-blood concentration ratio (µg eq/g of tissue per µg eq/ml blood) in rats, mice, rabbits and dogs at the same 50 mg/kg oral dose. Higher tissue-to-blood ratios (>1.0) were noted in the liver, kidneys and lungs in rats, mice and rabbits and in the spleen of mice. Lower ratios

Table V-8

Percentage of Administered ^{14}C-TNT (50 mg/kg)[a] Recovered from Various Organs 24 Hours After Oral Administration in Four Species

	Rats (3)[b,c]	Mice (8)[d]	Rabbits (2)[d]	Dog (1)[d]
Liver	0.4	0.4	0.6	1.5
Kidneys	0.2	<0.1	<0.1	0.1
Spleen	<0.1	<0.1	<0.1	0.3
Lungs	<0.1	<0.1	<0.1	0.1
Brain	<0.1	<0.1	<0.1	<0.1
Skeletal Muscle (as 40% body weight)	0.6	0.4	0.6	1.7
Whole Blood (as 7% body weight)	0.3	0.2	0.3	5.4

Based on El-hawari et al. (1981)

a/ Single dose of ring-UL-^{14}C-TNT
b/ (n)=numbers of animals analyzed
c/ Average recovery, male and females combined
d/ Males only

Table V-9

Tissue-to-Blood Concentration Ratios[a/] at 24 Hours After Oral Administration of ^{14}C-TNT (50 mg/kg) to Male Animals

	Rats	Mice	Rabbits	Dogs
Liver	4.1	5.2	3.8	0.8
Kidneys	3.3	3.3	1.6	0.3
Spleen	0.6	1.2	0.5	0.7
Lungs	1.2	1.7	1.1	0.3
Brain	0.3	0.4	0.2	<0.1
Muscle	0.5	0.5	0.3	<0.1
Fat	0.6	0.8	0.8	0.2
Blood	1.0(1.77)[b/]	1.0(0.93)	1.0(2.26)	1.0(29.22)

Based on El-hawari et al. (1981)

a/ µg eq/g tissue per µg eq/ml blood
b/ (n)=µg eq/ml

(<1.0) were generally noted in brain, muscle and fat of all species, spleen of rats and rabbits, and in all tissues of the dog. The results of this comparison seem to indicate that the unusually high blood concentratons, in µg eq/ml, may-account for the distribution pattern in dogs dosed at 50 mg/kg. When dosed at 5 mg/kg, the distribution pattern in various tissues of the dog indicates a higher percentage of radioactivity in the liver (2.4%), but a lower percentage (1.1%) in the blood. Comparison of the tissue-to-blood concentration ratios at this lower dosage level indicates values similar to those of the other species. Table V-10 compares these two dosing levels in dogs.

Radioactivity remaining in most tissues was comparable after oral and dermal administration, however, residual radioactivity was higher in the fat of all species following dermal application. The ^{14}C content of the liver was generally higher after oral dosing.

In general, radioactivity in most tissues of the rat was higher four hours after intratracheal instillation than after oral administration. The highest amounts of recovered radioactivity (1-8%) were found in muscle, blood and liver. In this study, levels in the muscle and blood of female rats were about two times higher than in males for both routes.

C. Excretion

Lee et al. (1975) measured the excretion of ^{14}C-labelled TNT in Charles River CD female rats. At 24 hours after dosing, an average of 53% of the administered dose was excreted in the urine while approximately 21% was found in the GI tract plus contents and 6% in the feces. Only negligible amounts were recovered in expired air.

Hodgson et al. (1977) and El-hawari et al. (1981) also studied the excretion of ^{14}C-labelled TNT in male and female Charles River CD or Sprague-Dawley rats. About 53% to 65% of the administered radioactivity was recovered in the urine in 24 hours while an average of 30% to 34% of the dose was found in the GI tract plus contents. Males and females excreted approximately 8% and 2%, respectively, in the feces.

In similar studies in albino CD1 mice, New Zealand rabbits and beagle dogs, Hodgson et al. (1977) and El-hawari et al. (1981) reported that excretion of radioactivity in mice was 42% to 60% in the urine, 9% to 43% in the feces and 7% to 13% in the GI tract plus contents (Table V-2).

In rabbits, these same authors reported the 24 hour excretion of the majority of the radioactivity in the urine (66% to 79%) with much smaller amounts in the GI tract plus contents (5% to 11%) and feces (<2%) (Table V-3).

Similar to rabbits, dogs excreted 56% to 60% of the radioactivity in the urine

Table V-10

Percentage of Orally Administered ^{14}C-TNT Recovered and Tissue-to-Blood Concentration Ratios in Male Dogs

	% Recovery		Tissue-to-Blood Concentration Ratios[a/]	
	5 mg/kg	50 mg/kg	5 mg/kg	50 mg/kg
Liver	2.4	1.5	4.9	0.8
Kidneys	0.1	0.1	1.4	0.3
Spleen	<0.1	0.3	0.8	0.7
Lungs	0.1	0.1	1.1	0.3
Brain	<0.1	<0.1	0.2	<0.1
Muscle (40% of body wt.)	1.4	1.7	0.2	<0.1
Blood (7% of body wt.)	1.1	5.4	1.0(0.72)[b]	1.0(29.22)
Fat	—	--	0.2	0.2

Based on El-hawari et al. (1981)

a/ µg eq/g tissue per µg eq/ml blood

b/ (n)=µg eq/ml

24 hours after oral dosing while the GI tract plus contents contained 4% to 10% and the feces 5% to 17% (Table V-4) (Hodgson et al., 1977; El-hawari et al., 1981).

When El-hawari et al. (1981) compared the 24 hour recovery of radiolabelled TNT in all four species following a single oral dose of 50 mg/kg to each, rats, mice and dogs excreted relatively equivalent amounts in the urine, averaging 60% of the dose while recovery from the urine of rabbits was approximately 75% of the dose. The percentage recovered from the feces and the GI tract plus contents was somewhat more variable between the species as shown in Table V-5.

El-hawari et al. (1981) also compared the excretion of TNT in rats, mice, rabbits and dogs as a function of route of administration. In all species, total excretion 24 hours after administration was lower after dermal application as compared to the oral route. When the oral route was compared to intratracheal instillation, 4 hour recovery was highest in the GI tract plus contents of the orally treated rats (76%) while similar amounts (approximately 15%) were recovered in the urine and GI tract plus contents after intratracheal instillation. A direct comparison between the dermal and intratracheal routes was not studied.

In this series of studies, El-hawari et al. (1981) reported the presence of a bright red pigment in the urine of rats and mice treated by different routes of administration while the urine of rabbits and dogs treated orally and dermally contained no such colored pigment, even after doses up to 50 mg/kg.

D. Metabolism

The presence of four functional groups on the TNT molecule would permit a variety of metabolic transformations to occur, to include oxidation of the methyl group, oxidation of the benzene nucleus, reduction of the nitro groups and conjugation. Only minute quantities of unmetabolized TNT have been identified in the urine, and *in vitro* experiments suggest that the liver is the major site of TNT biotransformation.

In the study by Lee et al. (1975), a thin-layer chromatographic (TLC) analysis of the radioactive compounds in the urine and in brain of rats using a solvent system of ethyl acetate:petroleum ether indicated that all the radioactivity in the 30-minute and 24-hour urine samples remained at the origin (point of application), whereas TNT has an R_f value (ratio of movement from origin:solvent front) of about 0.75 in this system. The radioactivity from the 30-minute brain extract had an R_f of 0.2, indicating the presence of one metabolite different from that in the urine. A 24-hour urine sample was also developed in a butanol:methanol:water system. There were one major and several minor peaks of radioactivity. Almost no radioactivity was associated with the TNT or the dinitrotoluenes which have R_f values of about 0.95 in this solvent system. No attempts were made in this study to identify TNT metabolites.

Hodgson et al. (1977) stated that analysis of urinary metabolites recovered indicated that TNT was metabolized extensively in all four species studied and similarly in rats, mice and dogs. The rabbit, on the other hand, had a somewhat different metabolic profile, suggesting a species difference in the metabolism of TNT. The presence of a red pigment in the urine of rats and mice, but not rabbits and dogs, also suggests species differences in metabolism. Furthermore, glucuronide conjugation appears to play an important role in the metabolism of TNT in the rat and dog, but not the rabbit.

In this study, initial TLC analysis was carried out on rat urine that had been extracted with $CHCl_3$:MeOH (3:1, v/v) both before and after hydrolysis with 5N HCl (at 100°C for 1 hour) and then evaporated and assayed for radioactivity. Metabolites identified in rat urine, with and without hydrolysis, included trinitrobenzyl alcohol, 4-amino-2,6- dinitrotoluene, 2-amino-4,6-dinitrotoluene, diaminonitrotoluenes (not specified) and trinitrobenzoic acid (in hydrolyzed urine only). Unmetabolized TNT accounted for <1% of urinary radioactivity. Urinary metabolites from other species were not presented.

In the study by El-hawari et al. (1981), urinary metabolites of rats, mice, dogs and rabbits were extracted with ethyl acetate under mildly acidic conditions and separated by thin layer chromatography using two solvent systems with different polarity. Tentative identification of metabolites was carried out by comparing solubility characteristics, reactions with specific spraying reagents, and R_f values of the metabolites with those of standard reference compounds. Quantitative determination of individual metabolites was not feasible in this study.

The authors stated that TNT was metabolized extensively in all species examined, whether treatment was oral, dermal or intratracheal. Large portions of the metabolic products were conjugated with glucuronic acid, but no conjugation with sulfates was indicated by incubation with aryl sulfatase. Most of the metabolic products were reduction derivatives, including the 2- and 4-hydroxylamines, the 2- and 4-monoaminodinitro and the 2,6- and 4,6-diaminomononitro derivatives. The trinitrobenzyl alcohol and the trinitrobenzoic acid seemed to be present, but confirmation was not possible. The parent compound, TNT, was detected in the urine of some species in minute quantities only. The extraction procedures used minimized the alterations of the hydroxylamines to azoxytoluene, but some of the latter was present, especially after fractionation of the urinary products in the presence of NaOH. Glucuronide conjugation appeared to play an important role in TNT metabolism, but while the amount of extractable radioactivity increased after incubation with β-glucuronidase, the TLC profiles remained unchanged. Other products of TNT metabolism remained unidentified. The metabolic profiles of urine from rats, mice, and dogs differed only quantitatively.

The urine of rats contained large amounts of the 4,6-diamine (and, to a lesser extent, the 2,6-diamine) and monoamines (the 4-amino and/or 2-amino). The 2- and 4-hydroxylamines and some azoxytoluene (probably formed during fractionation) were present in small quantities. Metabolic profiles of urine from male and female rats showed no significant differences. The amounts of glucuronides in urine collected from bile duct cannulated rats were lower than those collected from intact rats. In addition, the 4-hour urine from cannulated rats contained more of the polar metabolites and more parent TNT. Only minimal differences were apparent between orally and dermally treated rats (more unchanged TNT eliminated after dermal application) and quantitative differences between orally and intratracheally treated rats. The source of the red pigment was not identified.

Compared with rat urine, mouse urine contained smaller quantities of the polar metabolites and the diamines and more of the monoamines and hydroxylamines. Mouse urine also contained considerable amounts of the trinitrobenzyl alcohol and trinitrobenzoic acid. The presence of azoxytoluene was demonstrated after fractionation by acid or base. Urine of mice contained the least glucuronide. No major differences were evident after oral or dermal treatment except for the presence of larger quantities of unchanged TNT after dermal exposure.

The metabolic profiles of dog urine indicated the presence of appreciable amounts of diamines and monoamines. Only small amounts of the 4-hydroxylamine and 2-hydroxylamine, and minute amounts of azoxytoluene (which seemed to be formed during fractionation) were present. Smaller amounts of polar metabolites and larger amounts of parent TNT were demonstrated in the urine of dermally treated dogs when compared to those treated orally.

Rabbit urine showed a unique profile which differed quantitatively, and probably qualitatively, from that of rats, mice, and dogs. The presence of larger quantities of monoamines and hydroxylamines was demonstrated, in addition to either or both of the diamines. The presence of trinitrobenzyl alcohol and trinitrobenzoic acid was indicated by their R_f positions. TNT and the azoxytoluene were absent from fresh urine, but some of the latter was formed during fractionation with ether in the presence of NaOH. Urine from dermally treated rabbits differed quantitatively from that of orally treated animals with a sharp decrease in polar metabolites and some increases in the monoamines, hydroxylamines and azoxytoluene in the dermal treatment group.

These studies by El-hawari et al. (1981) indicate that TNT is extensively metabolized in all four species regardless of the route of exposure. The majority of urinary metabolites are of high polarity with very low extractability in organic solvents and conjugation with glucuronic acid is indicated. Most of the metabolic products are reduction derivatives and include the hydroxylamines and mono/diamino-dinitro derivatives. The presence of benzyl alcohol and acid are indicated but not confirmed. Parent TNT is present in the urine of only some of the species but only in minute quantities, although dermal administration seems to increase the excretion of unchanged TNT. The azoxytoluene appears to be formed during the extraction procedure in the presence of alkali. Identification of the source of the red pigment in rat and mouse urine was not successful.

Most differences in the metabolic profiles were quantitative in nature and were demonstrated both within and between species. Quantitative differences between oral and dermal treatment groups were minimal, being largely evidenced by an increased amount of unchanged TNT present after dermal administration. Conversely, major quantitative differences were demonstrated between orally and intratracheally treated animals.

Excretion patterns from rabbit urine presented a somewhat unique profile which was considered by the authors to most closely approximate that reported in the literature for humans. Even this dissimilarity from other animal species appeared mostly quantitative in nature, differing primarily in the amounts of hydroxylamines, which are present in the rabbit urine in larger quantities than in the other three animal species. Of significance may be the fact that only the urine of rats and mice contained a red pigment similar to that found in human urine after exposure to TNT.

VI. HEALTH EFFECTS

Health effects data from human occupational exposure to TNT and from laboratory experiments with animals administered TNT are summarized in this section. Lesions have been observed in many tissues and organ systems including brain, liver, blood, reproductive organs, kidneys, urinary bladder and eyes. Evidence is presented that TNT is both mutagenic and carcinogenic in bacterial and animal tests, respectively.

A. Health Effects in Humans

With the advent of the large scale manufacture of TNT during World War I, many munitions workers reportedly died of TNT intoxication. During one 7 month period, 475 deaths (2.8%) occurred among 17,000 cases of TNT poisoning. In one munitions plant alone, 105 fatalities (1.5%) occurred among 7,000 cases of TNT intoxication during a 20 month period (Zakhari and Villaume, 1978). Overall, in the four year period between 1914 and 1918, 580 deaths (2.4%) were reported in the United States out of 24,000 cases of known TNT poisonings (Rosenblatt, 1980). In British ammunition plants, 125 deaths (26.3%) over a 25 year period were reported among 475 cases of toxic jaundice resulting from exposure to TNT (Zakhari and Villaume, 1978).

With the increased awareness of the hazards of TNT exposure, the number of fatalities significantly decreased during World War II, despite a dramatic increase in the production of this explosive. Only 22 fatalities were reported in the period between June, 1941 and September, 1945 among all government-owned ordnance explosives plants. Eight (36%) were due to toxic hepatitis and 13 (59%) were due to aplastic anemia (Zakhari and Villaume, 1978). Only one-third of the 22 were exposed to TNT at average concentrations over 1.5 mg/m^3, the existing workplace standard (OSHA, 1981). Among these cases, hepatitis was reported to occur more frequently among younger persons (average age, 30 years), with aplastic anemia being the cause of death among older persons (average age, 45 years). The pathologic findings in the clinical hepatitis cases invariably included degenerative damage to the liver, usually accompanied by a great reduction in size and weight (NRC, 1982).

Since World War II, only occasional deaths due to TNT exposure have been reported and very few problems related to TNT use have been found in the English-language literature (Morton et al., 1976).

In an extensive review of the literature, Zakhari and Villaume (1978) reported on the various signs and symptoms of TNT toxicity and provided detailed descriptions of the more specific effects of TNT on individual body systems. The following is a summary of this report.

Initial exposure to TNT in the atmosphere may result in mild irritation of the respiratory passages (nasal discomfort, sneezing, epistaxis and rhinitis possibly associated with headache) and skin (erythema and papular eruptions progressing to desquamation and exfoliation). Gastrointestinal disorders, to include nausea, anorexia and constipation, sometimes associated with tightening of the chest, are among the first signs of possible intoxication. Epigastric pain not associated with food intake is a cardinal symptom.

Absorption of sufficient amounts of TNT through the skin or lungs can produce signs of cyanosis (due to methemoglobin formation), toxic jaundice (due to severe liver damage), aplastic anemia (due to damage to the erythropoietic system), cataract formation (possibly a direct effect of TNT vapor or dust; may be first and only clinical manifestation), menstrual disorders (hypo- or hypermenorrhea), neurological manifestations (neurasthenia, nystagmus, irregularities of tendon reflexes and adiadochokinesia; only 2.2% of the cases in one study manifested diffuse brain lesions; 50% of the persons examined in another study showed irregularities in their thermoregulating reaction to heat and cold (Kaganov et al., 1970 as cited in Zakhari and Villaume, 1978)) and nephrotoxicity (as evidenced by a significant rise in glomerular filtration rate, sodium retention, urgency, frequent micturition and lumbar pain).

Upon physical examination, the findings may include a yellow discoloration of the skin, nails and hair. This is usually due solely to staining with TNT and is not to be confused with the jaundice associated with liver toxicity. More significant would be a bluish discoloration of the mucosa indicative of developing cyanosis. Other physical findings might include dermatitis with or without rash (early appearing rashes may clear), epigastric pain, tenderness and/or spasm, enlarged and palpable liver and changes to the electrocardiogram (bradycardia, decreased amplitude of QRS complex, flattened T-wave) and electroencephalogram (decreased amplitude of biopotentials, slowed activity, poor reaction to stimuli), functional in nature, and apparently due to vascular disturbances in the brain (Ermakov et al., 1969 as cited in Zakhari and Villaume, 1978).

Laboratory findings include an amber to deep red coloration of the urine and various effects on the hematological parameters and blood chemistries. In several cases where TNT exposure resulted in death, specific post-mortem findings included fatty changes in the liver and kidneys. Foulerton (1918) as cited in Zakhari and Villaume (1978) reported that in 3 specific cases of death due to TNT intoxication (exposure level and duration not specified), the liver showed signs of advanced degeneration, disintegration of parenchyma, fibrosis and advanced interlobular round-cell infiltration. Fat was distributed in both parenchyma and fibrotic tissue. The kidney also showed signs of fat accumulation along with cloudy degeneration of the epithelium of the convoluted tubules. The glomeruli were, however, free of fat globules. Numerous fat granules were scattered throughout the interalveolar tissues of the lungs. Masses of brownish material were found in all three organ systems.

While there have been only limited reports in the English literature of cataract formation resulting from industrial exposure to TNT, Zakhari and Villaume (1978) described several studies that reported the finding of cataracts among European and Russian dynamite workers. The cataracts have been reported to often occur without other toxic manifestations (Manoilova, 1968) while Tyukina (1967) described changes in the crystalline lens as occurring in four stages and being characteristic of TNT-induced opacities, easily distinguishable from those of different origins. Hassman and Juran (1968) reported the occurrence of cataracts in 26/61 (42.6%) workers, average age of 44.5 years, exposed to TNT for an average of 8.4 years. The cataracts were described as V-shaped or lunar, white-grey in color and located in the area of the lens equator. In some cases, the opacities had merged to form an irregular ring. While atmospheric levels were not reported, the authors indicated that cataract formation was not associated with other toxic effects, and that repeated examinations indicated no other health effects in 26.9% of the workers with TNT-related cataracts. In 1978, Hassman et al. confirmed the occurrence of cataracts characteristic of TNT exposure in 87% of a group of 54 TNT workers with previously diagnosed or suspected TNT cataracts. Control subjects were not included in this study. Average exposure duration was approximately 14 years. Other TNT-related effects were minimal, confirmed in only 9% of the exposed group and reported as chronic TNT intoxication.

More recently, Harkonen et al. (1983) reported on the occurrence of equatorial lens opacities in 6 of 12 occupationally exposed workers in Finland. The opacities were described as bilateral and symmetrical. They had no effect on visual acuity or visual fields. They were detectable only in the periphery of the lens, being either continuous or discontinuous. Exposure duration was approximately 6.8 years with workroom air concentrations averaging 0.3 mg/m^3 with a range of 0.14 to 0.58 mg/m^3. Physical examination as well as several blood chemistry parameters were normal. The average age for the 12 workers was 39.5 years with the subgroup having positive cataract findings averaging 43.8 years vs 35.2 years in those without cataracts. In 1984, Makitie et al. reported that 18/21 (85%) workers exposed to TNT for a mean of 12.3 years in the processing and packing of explosives had detectable equatorial lens opacities, most frequently in the anterior cortex of the lens with decreasing density toward central areas. The mean age of the exposed workers was 41.1 years while atmospheric levels ranged from 0.1 to 0.4 mg/m^3. Ten workers showed varying degrees of central opacity, from minute spots to small rosettes, but these opacities were so slight that no effect was detectable on visual acuity. In 50% of those with the peripheral lens opacities, the density was so slight that no shadow was seen in fundus reflex photography. There have been no reports in the literature nor in occupational health surveys on the occurrence of cataracts in munitions workers in the United States.

The mechanism of TNT-cataract formation is not clearly defined. While more recent studies (Harkonen et al., 1983) have investigated radical formation, based upon the vulnerability of the peripheral lens fibers to effects of peroxidation, as a possible cause of TNT-related cataracts, no definitive conclusions could be drawn from this investigation. Several studies implicate direct contact and local absorption as the probable cause (Kroll and Kolevatykh, 1965; Manoilova, 1967 as cited in Zakhari and Villaume, 1978), based upon the absence of systemic effects in the majority of the exposed individuals with the positive cataract findings. The weak polarity of TNT also supports its ability to directly penetrate the lens.

It has also been found that individuals deficient in glucose-6-phosphate dehydrogenase (G6PD) may be particularly susceptible to TNT intoxication. In one report (Djerassi and Vitany, 1975 as cited in Zakhari and Villaume, 1978), onset of hemolytic episodes occurred in 3 individuals within 2 to 4 days after initial exposure to TNT. Based on these and similar findings, it was recommended that determination of G6PD activity be made a pre-employment requirement for TNT workers.

Effects on the white blood cells (WBCs), as evidenced by an increase in the large mononuclear leukocyte count, may also be an early indicator of TNT poisoning. Hamilton (1946) reported that increases in these cells usually preceeded symptoms of illness and levels remained elevated for 2 to 3 months following initial occurrence (cited in Zakhari and Villaume, 1978).

Toxic hepatitis and aplastic anemia have been reported as the principal cause of death following TNT intoxication. Zakhari and Villaume (1978) reported that several fatal cases of aplastic anemia were associated with earlier episodes of non-fatal toxic jaundice or hepatitis. They further indicated that aplastic anemia can occur after a latent period of several years following an attack of toxic jaundice. Hyperplasia of the bone marrow is the first reaction of the hemapoietic tissues to TNT poisoning.

In a report prepared by the Department of the Army, as guidance standards in industrial medicine and hygiene (DARCOM, 1976), gastrointestinal symptoms were reported as often the first indication of toxicity. This report also indicated the lack of a clear relationship between the occurrence of the dermatitis often associated with exposure to TNT and the development of systemic effects; either may exist in the absence of the other.

Older reports on the adverse health effects associated with exposure to TNT generally did not include information on workplace concentrations. In one uncontrolled study, Ermakov et al. (1969) as cited in NRC (1982), reported that 122 (21%) of 574 employees exposed to an average TNT concentration of 1 mg/m^3 were chronically poisoned; work exposures ranged from 6 to 25 years. Most of those affected had functional disorders of the central nervous system, with 22% (27) having chronic anemia and leukopenia, 20% (24) with cataracts, and 12% (15) with symptoms of hepatitis. No comparisons were made with unexposed control populations.

Several reports of controlled studies have provided some information early and subclinical effects of TNT exposure (Stewart et al., 1945, Ghawabi et al., 1974, and Hathaway, 1974 as cited in NRC, 1982; Morton et al., 1976). A significant finding in these epidemiologic studies is the occurrence of hematologic and hepatic abnormalities at TNT concentrations well below the Permissible Exposure Limit (PEL) of 1.5 mg/m^3 (OSHA, 1981). Among the most persistent findings were mild reductions in hematocrit (Hct), hemoglobin (Hgb) concentrations and red blood cell (RBC) counts of exposed persons. These findings have been attributed mostly to the destruction of red cells by hemolysis due to exposure to TNT or to its metabolites (Voegtlin et al., 1922, Cone, 1944, as cited in NRC, 1982; Hathaway, 1977).

In one study cited by Zakhari and Villaume (1978), a group of 62 undergraduate students were exposed to atmospheric concentrations of TNT ranging from 0.3 to 1.3 mg/m^3 for approximately 33 days (Stewart et al., 1945). Observed changes in 20% or more of the subjects included a decrease in Hgb and circulating blood cells, an increase in the number of reticulocytes, a small but significant decrease in plasma proteins and a significant increase in bilirubin. The authors indicated that males were more susceptible to the hemolytic effects of TNT than were females.

Goodwin (1972) reported that, in a 1951 study at the Lone Star Army Ammunition Plant (LSAAP) in Texarkana, Texas, mean atmospheric contaminant levels for TNT (dust and fumes) were 2.38 mg/m^3, with no exhaust ventilation systems in use. In a series of tests conducted under a Physical Recheck Examination Program, the Thymol Turbidity test, indicative of liver cell irritation, was used to evaluate liver impairment. From a total of 1,537 tests run during one screening period, 87.5% of the workers were within the selected normal range (to 2.9 MacLagen units) with no signs of liver toxicity. Of the remaining workers with liver function tests above the normal range, from 2.9 to >5 MacLagen units, 36 (<2.5%) showed classical symptoms of liver damage. Liver function values in the affected workers, initially >5 MacLagen units, returned to normal limits within three weeks of their removal from the contaminated environment.

In an occupational health study conducted by the U.S. Army Environmental Hygiene Agency (USAEHA) at a TNT washout facility at Letterkenny Army Depot in Pennsylvania, Friedlander et al. (1974) reported that employees exposed for 6 months to TNT at various work locations in the facility and at atmospheric levels ranging from <0.02 to 3.00+ mg/m^3 displayed clinically and statistically significant decreases in Hgb and Hct levels when compared to pre-exposure values. Furthermore, a statistical comparison of these post-exposure values with those of matched controls (non-exposed individuals) at the same facility indicated a higher rate of abnormalities in the exposed individuals and mean value differences between the two groups.

In addition to significant differences in the Hgb and Hct values ($0.005 \leq p \leq$

0.01), significant differences were also found in RBC count and blood urea nitrogen (BUN) ($0.005 \leq p \leq 0.01$) and in reticulocytes, eosinophils and glucose ($0.01 \leq p \leq 0.05$). No significant differences could be demonstrated in several other laboratory parameters including serum glutamic-oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH), serum alkaline phosphatase (SAP), cholesterol and total bilirubin, among others. It could not be determined from this report if the positive clinical findings were dose dependent.

In another occupational health survey (Morton and Ranadive, 1974) conducted by USAEHA at the Newport Army Ammunition Plant (NAAP), Indiana, the distribution of abnormal values among workers correlated closely with both an increased production rate (from 75% to >100% capacity) and an increase in TNT dust levels (from 0.3 mg/m^3 to 0.8 mg/m^3). Various parameters were tested including Hgb, SGOT and LDH. Based on the measured values, 62.8% of the TNT exposed individuals demonstrated abnormal findings. The detection rate (ability to identify abnormal results) ranged from approximately 26% when only Hgb values were evaluated to 100% when the values for all 3 parameters (Hgb, SGOT and LDH) were assessed. Recovery to normal levels occurred upon removal of the individual from sources of exposure but the time required for recovery could not be determined from the available data. No statistically significant differences could be found in the incidence of abnormalities when results were compared as to sex, age or race, but sampling size may not have been sufficient.

Further statistical analysis of these clinical parameters as measured prior to the time of increased TNT production (atmospheric levels of 0.3 mg/m^3) paired with those one month after production was increased (atmospheric levels of 0.8 mg/m^3) indicated a statistically significant increase in LDH levels ($P < 0.005$) and SGOT levels ($P < 0.01$) following the increase in production rate. No such correlation was seen in hemoglobin levels (Morton et al., 1976). This increase in both the number of individuals with abnormal test results and the degree of the abnormality were correlated with the higher atmospheric levels of TNT, leading the authors to question the suitability of the Threshold Limit Value (TLV) of 1.5 mg/m^3 recommended at that time (ACGIH, 1971).

In a follow-up to the two previously cited occupational health surveys at Army facilities, USAEHA performed a cross-sectional epidemiological study involving 626 employees exposed to one or more munition compounds (TNT, RDX[a/], HMX[b/]) and 865 non-exposed employees from 5 Army Ammunition Plants[c/] (Buck and Wilson, 1975). All individuals were evaluated for liver function (SAP, SGOT, serum glutamic-pyruvic transaminase (SGPT) and bilirubin) and hematological

a/ cyclotrimethylenetrinitramine (1 hexahydro-1,3,5-trinotro-1,3,5-triazine)

b/ cyclotetramethylenetetranitramine (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazoline)

c/ Joliet, Iowa, Milan, Volunteer and Holston

(Hgb, Hct and reticulocyte count) abnormalities. No evidence of liver toxicity was indicated by the parameters studied. This result appears to be in contrast to the positive findings of liver toxicity in the NAAP study. However, exposure levels in this cross-sectional study were generally <0.5 mg/m^3 with only approximately 12% of the TNT workers exposed at levels >0.5 mg/m^3 while at NAAP, exposure levels rose to approximately 0.8 mg/m^3. Accordingly, the authors indicated that 0.5 mg/m^3 may be considered a reasonable no effect level for hepatotoxicity.

On the other hand, a significant hematological effect was observed among TNT workers exposed in this cross-sectional study to atmospheric levels of <0.5 mg/m^3. This positive effect was evidenced by a dose response relationship for all three parameters and occurred more readily among males. These results suggested to the authors that low level TNT exposure (<0.5 mg/m^3) may induce a low grade hemolysis with a compensatory mild reticulocytosis. It was not possible to determine a no effect level for hematological effects from the study. As a result of this study, USAEHA recommended that the TLV for TNT in the work place be lowered from the existing level of 1.5 mg/m^3 to a level of 0.5 mg/m^3 and that the U.S. Army adopt 0.5 mg/m^3 as their airborne exposure standard for TNT.

B. Health Effects in Animal Experiments

1. Short-Term Exposure

As indicated by studies in rats, mice and dogs for periods up to four weeks, dietary intake of TNT resulted in early but not persistent decreases in body weight and food intake while the red pigmentation in the urine persisted throughout. Some anemia was evident but somewhat inconsistent while hemosiderosis of the spleen was seen in all three species. Rats developed testicular atrophy. Table VI-1 summarizes these toxicity studies.

Lee et al. (1975) determined the acute oral toxicity of TNT in Charles River CD rats and albino Swiss mice. Rats and mice were fasted for at least 16 hours prior to dosing by intragastric intubation with a 4.12% saturated solution of TNT in peanut oil. After treatment, the survivors were observed daily for 14 days for delayed mortality or toxic signs. The LD_{50} was calculated by a computer program based on the method of maximum likelihood of Finney (1971).

The acute LD_{50} values in male and female rats were 1,010 and 820 mg/kg, respectively; in male and female mice they were 1,014 and 1,009 mg/kg, respectively. Symmetrical coordinated convulsions associated with respiratory inhibition occurred within 5 to 15 minutes after dosing and continued for 1 to 2 hours. Death, when it occurred, was usually due to respiratory paralysis while survivors appeared cyanotic and exhibited ataxia. Recovery was complete in 24 to 48 hours. No gross pathology attributable to treatment was noted.

Table VI-1 Summary of Studies: Short-term Exposure of Animals to TNT

Reference	Species	Dose mg/kg/day[a]	Route	Duration weeks[a]
Lee et al. (1975)	rat, mouse	--	oral	LD_{50}
	rabbit	50%	dermal, ocular	72 hours
	guinea pig	4.12%	dermal	--
Newell et al. (1976)	rabbit	9% [b]	dermal, ocular	up to 7 days
	mouse	--	oral	LD_{50}
Dilley et al. (1978, 1982)	rat, mouse	--	oral	LD_{50}
	dog	0.2, 2.0, 20.0	oral	4
	rat (male)	1.8, 8.8, 42.7, 190.4	oral	4
	(female)	1.7, 8.5, 41.2, 180.4		
	mouse (male)	1.5, 7.0, 35.3, 184.3	oral	4
	(female)	1.5, 7.7, 35.9, 176.9		
Levine et al. (1984a)	mouse	0.3, 2.0, 14, 100, 700	oral	4

a/ Unless otherwise stated.
b/ Approximate %; wastewater residue.

Within 10 to 20 minutes after dosing, a bright red pigment which stained the fur and bedding appeared in the urine of both species and continued to be excreted for several hours after dosing.

Dilley et al. (1978, 1982) also conducted acute toxicity tests of TNT using immature Sprague Dawley-derived rats and Swiss Webster mice. The acute oral LD_{50}s were determined in animals that were fasted for at least 16 hours before they were dosed. Four or five dose levels with 10 males and 10 females per dose were used. The test material was administered as a suspension in corn oil via oral-dosing needles at a volume of 1 ml/100 g body weight. Observation of survivors continued for 2 weeks and any toxic signs were noted. All deaths were recorded and a gross examination was performed. The LD_{50} was calculated by the same method of Finney (1971).

The acute oral LD_{50}s of TNT were 660 mg/kg in both male and female mice and 1320 and 794 mg/kg in male and female rats, respectively. Initial toxic signs included inactivity and tremors within the first 1 or 2 hours, followed by petit mal convulsions. Red urine was noted in both species within 60 minutes after dosing. Death, when it occurred, was within 4 hours. Animals that survived the convulsions recovered and were still alive at 14 days after treatment.

The acute toxicological effects of actual and synthetic TNT wastewaters were reported by Newell et al. (1976) and Sasmore et al. (1977) before and after irradiation and at different pH values. The oral LD_{50} values of the lyophilized, reconstituted residues were determined in fasted, adult male and female Swiss-Webster mice (15-20 g). Authentic TNT wastewaters were obtained from the Joliet Army Ammunitions Plant (JAAP) load and pack (LAP) operations and initially contained 125.5 ppm TNT and 30 ppm RDX plus several minor, unidentified components. The LAP wastewater was lyophilized and reconstituted with water and TNT was added to give the expected 9% level (some TNT was lost under conditions of large-scale lyophilization). The bulk of the lyophilized residue (91%) was inorganic salts. The reconstituted solutions were adjusted for specific pH levels, photolyzed as specified and either lyophilized to solid residues or extracted with benzene and lyophilized. The residues were administered as corn oil suspensions/solutions. Table VI-2 compares the results of this toxicological evaluation. For the synthetic preparation, the degree, if any, of irradiation could not be accurately determined from the available data; pH was stated to be 7.0; lyophilized residues were administered in corn oil. The pure TNT samples were originally dissolved in distilled water.

Toxic signs following oral administration included lassitude, cyanosis, occasional muscular twitching, convulsions and red urine. Death occurred in the first 24 hours or not at all. Recovery was complete in 2-3 days. No gross pathological lesions were reported for the surviving animals autopsied after the 14-day observation period.

Table VI-2

Acute Oral LD_{50} (mg/kg) of TNT Wastewater Residue and Synthetic Preparation in Mice[a/]

	pH	Percentage Irradiation[b/] 0	50	100
JAAP residue (pink water)	5.0	--	1500	4900
	7.0	1300	2600	>5000
	9.4	--	1600	4200
JAAP residue (benzene fraction)[c/]	5.0	--		
	7.0	500		
	9.4	--		
JAAP residue (aqueous fraction)	5.0	--	4200	4700
	7.0	2500	4700	>5000
	9.4	--	3900	4400
TNT	7.0		830	
Synthetic Wastewater	7.0		250-280[d/]	

a/ Reference: Newell et al. (1976); residue administered as corn oil suspensions/solutions; LD_{50} calculated using method of Litchfield and Wilcoxon.

b/ Photolyzed until initial TNT concentrations decreased by 50% and nearly 100%.

c/ TNT reported to be major component of this fraction.

d/ Result of 2 separate determinations; percent irradiation, if any, not clear for this evaluation.

From these studies, the authors concluded that the toxic component of the LAP wastewaters obtained from JAAP was mostly extractable with benzene and that UV irradiation also reduced the wastewater toxicity. They further postulated that TNT may be the principal toxic component as it constituted approximately 90% of the organic material in the LAP water and was removed from wastewater both by irradiation and benzene extraction.

Oral LD_{50} studies in other species were not available in the literature. The following lethal doses for TNT (oral LD_{Lo}, lowest dose reported to have caused death) were reported: rabbit, 500 mg/kg; rat, 700 mg/kg; cat, 1,850 mg/kg (Wyon, 1921 as cited in NRC, 1982).

a. Skin and Eye Irritation, Dermal Sensitization

The primary skin and eye irritation tests in rabbits, using the modified Draize method (Federal Register, 1974 as cited in Lee et al., 1975) indicated that TNT was a mild irritant to the skin but did not irritate the eye. A red stain, similar to that seen in the urine, appeared both on the skin and around the eye after application of a 50% TNT-peanut oil paste (Lee et al., 1975).

Topical application of a saturated 4.12% solution of TNT in peanut oil to the clipped skin of guinea pigs produced a 40% response and was considered a moderate sensitizing agent (method of Magnusson and Kligman, 1969 as cited in Lee et al., 1975).

Newell et al. (1976) also evaluated the skin and eye irritation potential of the JAAP LAP wastewater dry, powdered residues using the Draize method in adult male and female New Zealand white rabbits (2-3 kg). No skin irritation was observed when either the 0% or 100% irradiated neat (unextracted) residue was applied to the prepared site; however, considerable red skin staining occurred particularly when the 100% irradiated residue was applied.

No eye irritation was observed when the nonirradiated lyophilized wastewater residue was instilled and washed 30 seconds or 5 minutes after instillation. Irritation, including iritis and corneal opacity, was observed for up to 3 days when this residue remained in the eye for 24 hours. Irritation was nearly absent 4 days after instillation and recovery was complete by 7 days post-treatment.

b. Four-Week Studies

In a four-week range finding study, Levine et al. (1984a) fed TNT (~99% pure) mixed in ground Purina chow to groups of 10 B6C3F1 mice/sex at levels of 0.0, 0.3, 2.0, 14, 100 or 700 mg/kg/day. Animals were observed for clinical signs of toxicity, and body weight and food consumption were measured. Prior to sacrifice during Test Week 5, the animals were subjected to an extensive hematological and clinical chemisty evaluation . Animals were sacrificed by carbon dioxide anesthesia, major organs were weighed, and all organs were fixed for histological examination; bone marrow smears were prepared. Statistical analyses were accomplished using Analysis of Variance tests and Dunnett's t-test when necessary.

No significant clinical signs of toxicity nor mortality were observed. Mice receiving the 100 and 700 mg/kg/day doses had red-stained bedding. High-dosed mice showed a reduction in weight gain and/or loss in body weight throughout the study but food intake was increased in males receiving this dietary level. At 100 mg/kg/day, only occasional and slight decreases in body weight gain were recorded with no alteration in food intake.

The only significant changes in the hematological parameters were a decrease in WBC in males and an increase in platelets in females receiving the high dose. A dose-related increase in bilirubin was apparent in both sexes with increases of 25% and 50% at the 100 and 700 mg/kg/day dose levels, respectively. Weights of the kidneys and testes were significantly decreased in male mice at the 700 mg/kg/day level.

A diffuse increase in the relative amounts of yellow-brown pigment, resembling hemosiderin, was present in the red pulp of the spleen. The increase was of minimal severity at the 100 mg/kg/day level and of mild severity at the high-dose level. Extramedullary hematopoiesis did not differ in incidence from controls. No treatment-related lesions of the testes were evident in this study. No neoplastic lesions were observed. This study identifies a No-Observed-Adverse-Effect-Level (NOAEL) of 14 mg/kg/day for absence of effects on body weight, serum bilirubin and the spleen.

In four-week oral toxicity studies conducted by Dilley et al. (1978, 1982) in dogs, rats and mice, the appearance of red urine consistently occurred at the highest dose level in dogs and at the two highest dose levels in rats and mice. A decrease in weight gain accompanied by a decreased food intake occurred during the first week or two of treatment with recovery toward normal levels often occurring thereafter. Mild to moderate anemia and increased spleen weights, usually accompanied by hemosiderosis, at the high dose level were also common to all three species. Atrophy of the testes occurred in rats, and increased cholesterol levels and decreased SGPT activity were evident in dogs and rats.

In the dog study, 40 beagle dogs, approximately six months in age at the start of the experiment, were divided into four groups of five males and five females each. The treatment groups received a TNT/lactose mixture equivalent to 0.2, 2.0 or 20.0 mg/kg/day by capsule. The control group received lactose only, also by capsule. All dogs were observed for signs of toxicity and weighed weekly, and food intakes were recorded five days/week. Tests include extensive hematology, clinical chemistry and urinalysis. At the end of four weeks, one dog/sex/group was sacrificed and subjected to a complete histopathological examination. An additional dog/sex/group was removed from the treatment routine and placed on a recovery study. The remaining dogs were continued on the treatment regimen, and results are discussed under 13-week studies.

No toxic signs were reported for the dogs treated at 0.2 or 2.0 mg/kg/day. At 20 mg/kg/day, loose mucoid stools and diarrhea were frequently observed. Orange-tinted urine was evident by day six of treatment and continued to termination. Sporadic periods of inactivity occurred in males receiving the high-dose level. Both treatment and control groups lost weight during the first week of the study with the high-dose group showing the greatest degree of loss and the slowest recovery rate, but no statistically significant effects on body weight were detected. Food intakes were appreciably lower in the high-dose group during Week 1 and slightly lower during the second and third weeks. Organ weight analysis indicated an enlarged spleen in the male and an enlarged liver in the female sacrificed after four weeks of treatment with TNT at 20 mg/kg/day. Anemia, as evidenced by decreases in RBCs, Hgb, Hct and mean corpuscular hemoglobin concentration (MCHC) and an increase in mean corpuscular volume (MCV), was pronounced at the high-dose level with Hgb and MCHC values significantly decreased in both sexes. Clinical chemistry studies revealed statistically significant increases in cholesterol and bilirubin and decreases in SGPT and iron in dogs receiving 20 mg/kg/day but not all parameters were significant in both sexes. After the four week recovery period, cholesterol in males and bilirubin in females tended to remain elevated while SGPT values returned toward normal. In contrast, iron levels were greatly increased in both sexes after this recovery period. Histopathological examination revealed no clear cut treatment-related effects except possibly the hemosiderosis of the spleen, seemingly related to the anemia, in the female dog receiving 20 mg/kg/day for four weeks. A NOAEL of 2 mg/kg/day for absence of anemia and effects on the liver and spleen is indicated.

In the rat studies (Dilley et al., 1978, 1982), five groups of Sprague-Dawley rats, 20/sex/group, received 0%, 0.002%, 0.01%, 0.05% or 0.25% TNT mixed in powdered Purina Laboratory Chow (or approximately 0, 1.8, 8.8, 42.7 and 190.4 mg/kg/day of TNT, respectively, for males and 0, 1.7, 8.5, 41.2 and 180.4 mg/kg/day, respectively, for females, based on the authors' data for average intake of TNT over the four week period). All animals were observed for toxic signs; animal weight and food intake were determined weekly. Tests included hematology and clinical chemistry. At the end of four weeks of treatment, five rats/sex/level were fasted for 16 hours, anesthetized with chloroform, and blood was withdrawn by heart puncture. The animals were sacrificed, major organs were weighed and all organs were fixed for histological examination. Five additional rats/sex/level were removed from the treatment regimen and allowed to recover. The remaining rats were continued on the treatment regimen for 13 weeks, and results are described under "Longer-Term Exposure".

No toxic signs were apparent. Those rats receiving the two highest doses (0.05% and 0.25%) developed a red color in the urine from Day 2 continuously through the day of sacrifice. Body weights were significantly reduced in the high-dose group, as were food intakes. In the high-dose male rats, a significant increase was observed in the absolute and relative liver and spleen weights and a decrease in the weight of the testes. Only the spleens of these females were similarly affected although the liver to body weight ratios, but not the absolute liver weights, were increased. There were no statistically significant differences in hematological parameters after 4 weeks of treatment although RBC, Hgb, and Hct values were reduced in both sexes receiving the high dose. Only cholesterol was significantly increased in both sexes at the high TNT dose. After four weeks of treatment, all males at the high dose (0.25%) displayed testicular atrophy and hyperplasia of the interstitial cells. One male at the 0.05% TNT dose also had testicular atrophy. Hemosiderosis of the spleen was present in all rats receiving the high dose and in one female receiving 0.05% TNT. No microscopic lesions associated with the gross hepatomegaly were found. No other lesions were found to be related to TNT treatment although the rats receiving the highest dose appeared to have greater susceptibility to respiratory disease, as evidenced by an increased frequency of alveolar collapse and dilation in the high-dose females.

During the four week recovery study, the red color disappeared from the urine in 15 days, weights increased toward normal levels, and food consumption increased in the high-dose groups. The weight of the testes from the high-dose males remained low but the anemia was absent and cholesterol values and SGPT activity were normal. Testicular atrophy and hyperplasia of the interstitial cells were present in all five high-dose males along with hemosiderosis of the spleen in four of the five females. Based on the absence of testicular affects and lesions in the spleen, a NOAEL of 8.8 mg/kg/day is indicated.

In the mouse studies (Dilley et al., 1978, 1982), 20 male and 20 female Swiss Webster mice per group received TNT in the diet at 0.0, 0.001, 0.005, 0.025 or 0.125% TNT by weight (equivalent to approximately 0, 1.5, 7.0, 35.3 and 184.3 mg/kg/day, respectively, in males, and 0, 1.5, 7.7, 35.9 and 176.9 mg/kg/day, respectively, in females). Weekly body weights and food consumption were determined. Hematological parameters were measured. At the end of four weeks of treatment, five mice/sex/level were sacrificed by anesthesia, major organs were weighed, and all organs were prepared for histopathological examination. Five additional mice/sex/level were placed on a four week recovery study. The remaining 10 mice/sex/level were continued on the study for a total of 13 weeks. Results are reported under "Longer-term Exposure".

No toxic signs related to TNT treatment were apparent. The urine of mice receiving the two highest dose levels (0.025% and 0.125%) became red in four to six days after the start of treatment and remained red throughout the treatment period. The color disappeared from the urine 10 days after the mice were placed on the recovery study. Body weights were significantly lower than controls in both sexes receiving 0.125% TNT after one week of treatment but recovery toward control levels occurred over the next several weeks. Decreased weight gain was also evident at the 0.025% level but not significantly so. Food consumption was decreased in both sexes at the two highest dose levels during the first week but was not significantly different from control intake; recovery to normal intakes occurred over the next two weeks. During the recovery period, the mice at the high-dose level slightly increased food consumption during the first week but body weights did not change to any notable degree. The only treatment-related effect on organ weight was a significant increase in the spleen-to-body weight ratio in the high-dose males. While not statistically significant, the mice receiving the high dose of TNT, particularly the females, showed signs of anemia, as evidenced by a decrease in RBC count, Hgb and Hct, and smaller increases in MCV, MCH and MCHC. After the four week recovery period, few signs of anemia were evident. No treatment-related effects were observed upon histological examination. A NOAEL of 35.3 mg/kg/day is based on marginal effects at the higher dose level on the spleen and hematopoietic system, both apparent target organs for TNT toxicity.

2. Longer-Term Exposure

In studies conducted in rats, mice, dogs and monkeys for periods ranging from 13 weeks to 2 years, dose-related reductions in body weight and food intake as well as anemia and red pigmented urine were evident in most species. Organs consistently affected by TNT intake include the liver and spleen in dogs, rats and mice as well as the testes in rats. No ophthalmic effects were evident. Evidence of carcinogenicity was established. Table VI-3 summarizes these toxicity studies.

a. Thirteen-Week Studies

In a continuation of the four-week study described under "Short-term Exposure", Dilley et al. (1978, 1982) exposed dogs, rats and mice to TNT for a total of 13 weeks. Groups of these animals were removed from the test diet and allowed to recover for an additional four weeks. A red coloration of the urine was common to all three species at the higher dose levels. Only rats showed a persistent negative effect of TNT treatment on body weight gain. Anemia was apparent in all three species while hemosiderosis of the spleen was seen predominantly in rats and mice. Liver weights were increased in dogs and mice with some necrosis in the mice. Cholesterol levels were increased and SGPT activity was decreased in dogs and rats; these parameters were not measured in mice. Treatment with TNT for 13 weeks had no apparent effect on survival in any of these species.

In the dog study, three males and three females per group were administered by

Table VI-3 Summary of Studies: Long-term Exposure of Animals to TNT

Reference	Species	Dose mg/kg/day[a]	Route	Duration weeks[a]
Dilley et al. (1978, 1982)	dog	0.2, 2.0, 20	oral	13
	rat (male)	1.4, 7.0, 34.7, 160	oral	13
	(female)	1.4, 7.4, 36.4, 164.4		
	mouse (male)	1.6, 7.5, 35.7, 193	oral	13
	(female)	1.6, 8.0, 37.8, 184.2		
Levine et al. (1981, 1984b)	rat	1, 5, 25, 125, 300	oral	13
Martin (1974)	monkey	0.02, 0.1, 1.0	oral	90 day
Hart (1974)	dog	0.02, 0.1, 1.0	oral	90 day
Levine (1983)	dog	0.5, 2, 8, 32	oral	26
Furedi et al. (1984a, b, c)	rat	0.4, 2, 10, 50	oral	104
(1984d, e, f)	mouse	1.5, 10, 70	oral	104

[a] Unless otherwise stated.

capsule 0.0, 0.2, 2.0 or 20 mg TNT/kg/day mixed with lactose for a total of 13 weeks at which time two dogs/sex/level were fasted overnight. The animals were anesthetized, and blood was withdrawn for hematological and chemical analysis. The animals were sacrificed, major organs were weighed, and all organs were fixed for histological examination. Body weights and food intakes were measured weekly. The remaining dog/sex/level was removed from the test regimen and allowed to recover for an additional four weeks.

The only gross signs of toxicity in the dogs were loose mucoid stools and diarrhea in the 20 mg/kg/day group. Periods of sporadic inactivity were evident in these males throughout the study, becoming persistent by Week 12 through termination of the treatment. On one occasion, one male displayed signs of nystagmus. Red colored urine appeared in all dogs on the high-dose level, beginning on Day 6 and persisting as long as TNT was administered. The color returned to normal during the recovery period. During Week 12, one male receiving 20 mg/kg/day became moribund and was sacrificed. Necropsy revealed swelling of the left upper cerebral hemisphere, lung congestion, hemorrhagic lymph nodes and enlarged kidneys, liver and spleen. Anemia was marked, and cholesterol and SAP were increased. The authors indicated that the presence of a duodenal nematode may have been a contributing factor in some of these lesions. No other dogs succumbed to the treatment regimen.

No statistically significant effects on overall body weight were evident although all groups lost some weight during the first week; high-dose females lost appreciably more weight than controls. Of note is the observation that the male and female placed in the recovery study after receiving TNT at 20 mg/kg/day for 13 weeks lost weight during this period, with the reversal in weight beginning around treatment Week 10 and continuing thereafter. This prompted the authors to speculate on a possible delayed onset of toxicity. Food intake was appreciably lower in the high-dose group during the first week and remained somewhat lower throughout. No other differences in intake could be related to treatment.

Absolute and relative liver and spleen weights were increased in both sexes receiving the high dose but both values were within normal range in the recovery group. While anemia was pronounced in both sexes on the high-dose level during the first four weeks of treatment (as indicated by significant decreases in Hgb, Hct and MCHC, along with an apparent decrease in RBC count), by Week 13, only the MCHC remained significantly depressed. These parameters were within normal range in the female dog on the four-week recovery regimen, but they appeared depressed in the high-dose male. Clinical chemistry tests showed a significant increase in bilirubin and creatinine and a significant decrease in SGPT and BUN in females receiving 20 mg/kg/day for 13 weeks. In the males, no significant differences occurred; however, bilirubin and cholesterol appeared appreciably increased while SGPT and BUN appeared similarly decreased. All parameters were within normal range in the recovery animals except for an apparently high cholesterol and low SGPT in the high-dose males.

Except for the notable pathology in the high-dose male sacrificed during Week 12 (as previously described), the only other lesions of possible significance were an enlargement and pigmentation of the parenchymal macrophages in the liver of the remaining male and one of the two females. This study establishes a NOAEL of 2.0 mg/kg/day for the absence of anemia along with the absence of toxic effects on the liver and spleen.

In the rat study, essentially a continuation of the four-week study by Dilley et al. (1978, 1982) previously described, groups of 10 male and 10 female Sprague-Dawley rats were exposed to TNT in the diet at 0%, 0.002%, 0.01%, 0.05% or 0.25% for a total of 13 weeks. These doses were equivalent to approximately 0, 1.4, 7.0, 34.7 and 160 mg/kg/day, respectively, for males and 0, 1.4, 7.4, 36.4 and 164.4 mg/kg/day, respectively, for females, based on an average calculated by the authors. Body weights and food intake were recorded weekly. At 13 weeks, five males and five females per dose level were anesthetized with chloroform, and blood was withdrawn by heart puncture for hematological and chemical analyses. The animals were sacrificed, major organs were weighed, and all organs were fixed for histological examination. The five remaining rats/sex/dose were removed from the test diets and allowed to recover for four weeks.

No deaths occurred before scheduled sacrifices and no signs of toxicity were observed at any dose level. All rats on the two highest dose levels (0.25% and 0.05%) developed a red color in the urine beginning on the second day of treatment. A similar color appeared on Day 50 in the urine of rats receiving 0.01%. This red color persisted through termination of treatment but disappeared after 16 days on the recovery study. Significant findings in the rat study at the high-dose level (0.25%) included growth suppression, beginning during the first week and continuing throughout (possible "systemic errors" in the weighing procedure was indicated for the two lowest dose levels during Week 9), accompanied by a decreased food intake, a significant decrease in the absolute weight of the kidney and absolute and relative weight of the testes in males and an increase in absolute and relative spleen weight in both sexes. Anemia, as evidenced by significant decreases in Hgb, Hct and MCHC, an increase in MCH, and an accompanying but non-significant decrease in RBC count, was present in both sexes receiving the high dose. Clinical chemistry values included a significant increase in uric acid and cholesterol and a decrease in glucose in males and females while males also showed significant decreases in SGPT, SAP and iron and an increase in creatinine.

At the next highest dose level (0.05%), signs of anemia were evidenced by significant decreases in Hgb and Hct in both sexes and RBC count in females. Female rats receiving 0.01% TNT also showed similar signs of anemia. Iron levels were significantly decreased in males receiving TNT at 0.05% and 0.01% but not at the lowest dose (0.002%). Glucose levels were significantly decreased in males at the 0.05% test level and in females at the two lowest levels (0.01% and 0.002%). Hemosiderosis of the spleen and interstitial lymphocytes in the kidney were the only lesions common to both sexes at the high-dose level and, to some degree, at the next highest level (0.25% and 0.05%, respectively) that were attributed to treatment with TNT (although two rats/sex at the control level also displayed some degree of hemosiderosis. This, however, became evident in controls only after four weeks on the extended recovery study.) Male rats on the high dose were reported to have atrophy of the epididymis and testes accompanied by hyperplasia of the interstitial cells. No histopathology was done on rats at the two lowest dose levels after 13-weeks of treatment, despite significant findings at the next highest level.

After four weeks of recovery, most of the above described parameters returned toward normal level; however, the Hgb, Hct and MCH in the high-dose males were significantly increased while the kidney and testicular weights remained significantly decreased. The liver, spleen and brain weights were significantly increased in females. Histological findings were similar to those after the 13 weeks of treatment. An unexplained finding at the three highest dose levels (0.01%, 0.05% and 0.25%) allowed to recover for four weeks was a significant decrease in albumin (A) to approximately one half of control levels and a significant and marked increase in globulin (G) levels to 6 to 10 times the control levels. This resulted in a dramatic reversal of the A/G ratio, 10 to 25 times higher than control levels. The authors discounted this finding as "inconsistent". A NOAEL of 1.4 mg/kg/day is indicated by the occurrence of anemia at the next highest treatment level.

Dilley et al. (1978, 1982) also continued 10 Swiss-Webster mice/sex/level on diets treated with TNT at levels of 0.0%, 0.001%, 0.005%, 0.025% or 0.125% for a total of 13 weeks. From intake and weight data, the average intake of TNT was calculated to correspond to approximately 0, 1.6, 7.5, 35.7 and 193.0 mg/kg/day, respectively, for males and 0, 1.6, 8.0, 37.8 and 184.2 mg/kg/day, respectively, for females. Animals were observed for signs of toxicity; body weight and food intake were recorded weekly. At the end of 13 weeks, the mice were fasted for 16 hours, anesthetized with chloroform, and blood was withdrawn by heart puncture for hematology. The mice were sacrificed, major organs were weighed, and all organs were fixed for histological examination. No toxic signs attributed to treatment were reported. Red color appeared in the urine of mice receiving the two highest doses (0.025% and 0.125%) within 4-6 days from the start of treatment and remained throughout the dosing period. Premature deaths included one male per group from the three highest dose levels (0.005%, 0.025%, 0.125%) during Week 2 and one additional male each at 0.025% and 0.005% during Weeks 6 and 13, respectively. No TNT treated females died during the study but one control died during Week 8. This attrition rate was not considered significant by the authors.

A significant decrease in body weight occurred in both sexes on the high-dose level during Week 1, but quickly returned to normal levels during the next few weeks. A slight but not significant decrease in food intake during this same period was probably indicative of an initial aversion to the diet. It should be noted that control females in this study exhibited an "abnormally low growth pattern" with low intake throughout. No significant effects were present in hematological parameters although males at the two highest levels showed a slight decrease in Hct. Heart and heart-to-brain weight ratios were significantly increased in males receiving 0.005% and 0.125% TNT but this finding did not correlate to any pathology. A slight but significant increase in absolute and relative spleen weight was evident in females receiving the high dose; all females at this level displayed a hemosiderosis of the spleen as did 3/5 males. The authors felt that this was the only treatment-related effect in mice receiving TNT for 13 weeks. Dilation and/or hyperplasia or edema of the uterus occurred in some TNT treated females at 13 weeks or after four weeks of recovery but was not statistically significant.

While chronic respiratory disease occurred in many mice at all levels in this study, male mice on the two highest dose levels had a greater incidence of complications, such as alveolar dilation and collapse. Significance of these findings is not known but could indicate an increased sensitivity to this condition.

In the mice treated for 13 weeks and allowed to recover for four additional weeks, the only significant findings were an increase in absolute and relative kidney and liver weight in high-dose males and in spleen weights of the females. Two of these males also displayed necrosis of the liver. Hemosiderosis of the spleen was evident in 80% to 100% of the mice on the high-dose level (0.125%) and 80% of the females receiving the 0.025% TNT level for 13 weeks with four weeks of recovery. A NOAEL of 1.6 mg/kg/day is indicated by premature deaths (2/10) along with effects on the heart weight in males at the next higher dose level.

Levine et al. (1981, 1984b) conducted a 13-week study in which Fischer 344 rats (10/sex/dose level) were administered TNT (~99% pure) in the diet at 1, 5, 25, 125, or 300 mg/kg/day. Thirty animals per sex were used as controls and received the same rodent chow used to prepare the test diets. Animals were observed daily for pharmacologic and/or toxic signs; test diets were changed weekly and prepared on the basis of weight and intake for each sex; clinical chemistry and hematology tests were performed following a 17-19 hour fast on all surviving animals during Test Week 13. At sacrifice, the brain, gonads, heart, kidneys, liver, and spleen were weighed, and all tissues were collected and fixed for histological examination.

Sacrifice of the surviving animals was accomplished over Weeks 14 and 15 of the study with one rat/sex/test group and three rats/sex/control group sacrificed each day. Test diets were administered up to approximately one day prior to the animals' sacrifice by carbon dioxide anesthesia. Control and high level test animals were subjected to a complete histopathological examination with all other levels limited to examination of those major organs that were routinely weighed. The authors further noted that since the male rats were received on site two weeks before the females, the study was conducted in two phases with the males always two weeks ahead of the females.

Clinical observations included lethargy and/or ataxia which were seen during Week 1 and to a lesser extent during subsequent test weeks for several animals receiving TNT. At Week 6, some of the males receiving TNT at 300 mg/kg/day were noted by palpation to have smaller than normal size testes. Red-stained bedding was seen at all TNT dose levels except for the lowest (1 mg/kg/day) level.

Administration of various levels of TNT in the diet did not appear to have an adverse effect on survival time. While one male and one female at the 300 mg/kg/day level died during Week 13 of the study, death occurred within days following blood collection and was postulated to be due to a combination of TNT induced anemia and the stress of a reduced blood volume.

Dose-related reductions in body weight gains were, in general, observed for male TNT-treated rats throughout the 13-week treatment period. These reductions amount to approximately 5% at the 1 mg/kg/day level, 10% at 5 and 25 mg/kg/day, 24% at 125 mg/kg/day and 46% at the 300 mg/kg/day level at Test Week 13. Although female body weight gains were largely unaffected at doses up to 25 mg/kg/day at Test Week 13, an approximate 27% reduction in body weight gain was observed at 125 mg/kg/day with approximately a 38% reduction at the 300 mg/kg/day level. These reductions in weight gain were significant ($p<0.05$) for both sexes at the 125 and 300 mg/kg/day treatment level throughout most of the experiment.

Dose-related decreases in food consumption were observed for TNT-treated rats. Significant reductions in food intake were evident for males and females receiving TNT at 125 or 300 mg/kg/day throughout most of the study. At Week 13, food intake for these two dose levels was reduced in females by 28% as compared to control intake, and in males by 6 and 23%, respectively.

Analysis of hematologic parameters indicated a dose-dependent anemia (decreased Hct, Hgb and RBCs) for TNT-treated rats. Males appeared to be slightly more sensitive than females, with the 25 mg/kg/day level and higher resulting in statistically significant reductions in these parameters. Only marginal decreases were apparent for female rats at the 25 mg/kg/day dose level with significant differences in these parameters at the two higher dose levels. Statistically significant methemoglobin production was observed in males and females receiving 300 mg/kg/day. Compensatory responses to the TNT-induced anemia included reticulocytosis, macrocytosis and elevated levels of nucleated erythrocytes. Mean corpuscular hemoglobin levels were slightly elevated at the 300 mg/kg/day level and macrocytic erythrocytes, seen only at 300 mg/kg/day, were marginally hypochromic.

In clinical chemistry measurements, a dose-dependent elevation of serum cholesterol levels was seen in TNT-treated rats. Cholesterol values for both sexes at 125 and 300 mg/kg/day were significantly increased ($p<0.05$) over control animals. Additionally, an indication of a TNT-induced elevation in serum triglycerides was seen in females at 300 mg/kg/day as evidenced by a statistically significant increase in this parameter of 28% over control values. While male rats receiving TNT at 125 mg/kg/day had a 34% elevation in this parameter, this increase was not statistically significant nor was a similar increase seen in males receiving 300 mg/kg/day of TNT.

A dose-related hepatomegaly was statistically significant at the 125 and 300 mg/kg/day levels in both sexes as was an increase in absolute and relative spleen weights in females at both levels and in males at the 300 mg/kg/day dose level. Significant testicular atrophy, first noted by palpation in Week 6 of this study, was observed at the 300 mg/kg/day and, to a much lesser extent, at the 125 mg/kg/day dose level. Relative kidney weights exhibited a slight but dose-related increase in both sexes while absolute heart weights were significantly decreased in both sexes at the 300 mg/kg/day treatment level.

Histopathologic examinations revealed brain lesions consisting of focal vacuolation and/or malacia of the white tracts of the cerebellar folia in six males and three females at the highest TNT dose level (300 mg/kg/day). One of these females died spontaneously at Week 13 following hematological blood sampling. The vacuolar lesions were described as small, oval, empty spaces of minimal severity and were observed in two animals per sex. The leukomalacia was reported to consist of a demyelination and to appear as a progression of the vacuolar lesions, manifested by aggregation of lipid-laden macrophages in the immediate vicinity of the vacuoles.

Varying degrees of either multifocal or diffuse hepatocellular hypertrophy (hepatocytomegaly), manifested as an increase in the cytoplasmic mass of those hepatocytes located in the centrilobular to midzonal regions of the affected lobules, were found in six males treated at 125 mg/kg/day, in all ten males and in eight of the females given TNT at 300 mg/kg/day. One male and six females at this high-dose level also exhibited a minimal degree of focal bile duct proliferation.

Most of the rats receiving 125 mg/kg/day of TNT manifested an increase in yellowish brown pigmentation in the tubular epithelial cell cytoplasm of the renal cortices. The kidneys of all animals at the 300 mg/kg/day dose level exhibited similar but more severe and diffuse lesions.

The splenomegaly observed by gross examination in some of the animals receiving 125 or 300 mg/kg/day and confirmed by organ weight data corresponded with a mild to moderate diffuse sinusoidal congestion found at both of these dose levels, as well as in one male at the 25 mg/kg/day level. In addition, minimal to mild increases in hemosiderin-like pigment were found in the macrophages of the splenic red pulp and liver at the high-dose level. These observations, along with the dose-related anemia, suggested to the authors that this TNT-induced anemia was hemolytic in origin. This concept was supported by a lack of bone marrow cytotoxicity and a slight methemoglobinemia at the 300 mg/kg/day dose level.

Histologic examination of the testes revealed a dose-related degeneration of the germinal epithelium lining the seminiferous tubules in one, six and ten males at 25, 125 and 300 mg/kg/day, respectively. At the 300 mg/kg/day dose level the testicular lesions were diffuse, bilateral and marked in severity. The minimal to mild lesions were characterized by a diminution of spermatozoa, spermatids, and spermatocytes as a result of degeneration and necrosis. Spermatocytic and spermatidic giant cells, present in the lumen of some affected tubules, appeared to represent an early degenerative stage of this lesion. Those animals with lesions of moderate severity exhibited an absence of spermatozoa and spermatids, with only a few spermatocytes remaining in the degenerative tubule. The Sertoli cells and spermatogonia appeared unaffected. Atrophic seminiferous tubules lined with a few Sertoli cells and spermatogonia characterized those lesions graded as marked in severity. All males at the highest TNT dose (300 mg/kg/day) also exhibited a mild to moderate diffuse hyperplasia of interstitial (Leydig) cells in both testes along with intertubular edema. A NOAEL of 5 mg/kg/day is indicated by the absence of testicular degeneration and effects on the spleen at this dose level.

Martin (1974) conducted a 90-day study to evaluate the toxicity of TNT administered by gastric intubation as a suspension in a 1% aqueous solution of methyl cellulose to cynomolgus monkeys at daily dosages of 0.02, 0.1, or 1.0 mg/kg/day. The animals ranged in age from 36 to 56 months and in weight from 2.0 to 4.2 kg for females and 2.6 to 4.6 kg for males, and groups consisted of three males and three females each. Controls received the aqueous solution of 1% methyl cellulose.

Daily observations were made to detect clinical signs of toxicity; body weights were recorded weekly. Prior to the start of the study and during Weeks 5 and 9 and at the termination of the test period, the authors conducted hematological and clinical chemistry determinations, urinalysis, bromosulfophthalein (BSP) clearance tests and determinations of plasma TNT levels. Ophthalmoscopic examinations were conducted prior to the study and again at the close. At necropsy, histological examinations of thyroid, heart, liver, kidneys, adrenal gland, stomach, small intestine, lung, spleen, bone marrow and brain were made. Because only three animals of each sex were used in each dosage group, statistical analysis of results was not conducted.

No clinical signs of toxicity were attributed to TNT administration, nor were there any consistent abnormalities in any of the clinical laboratory tests conducted. Histologic examination showed some increases in numbers of necrotic megakaryocytes in high-dose females and determined that two of these females showed no normal megakaryocytes in bone marrow sections. This condition was described as a toxic manifestation, possibly related to thrombocytopenia, but the lack of platelet counts resulted in an inability to confirm this suspicion. Increased amounts of iron-positive material in liver cord cytoplasm was found at the highest dosage of TNT (1.0 mg/kg/day). The authors stated that the toxicologic importance of these two findings is uncertain. A NOAEL or Lowest-Observed-Adverse-Effect-Level (LOAEL) could not be determined for this study due to the small numbers of animals evaluated along with the lack of statistical evaluation.

Hart (1974) also conducted a 90-day toxicity study in purebred beagle dogs administered TNT in the diet (consisting of ground dog chow supplemented with commercial canned dog food) at dosage levels of 0.02, 0.1, or 1 mg/kg/day. Three dogs per sex per dosage level were used. Controls received the normal ground dog chow mixed with canned dog food. Daily observations of toxic and pharmacologic effects were conducted, and animals were weighed weekly. Twice prior to the start of this study and during the 4th, 8th, and terminal weeks of the study, the authors conducted hematological and clinical biochemical tests, and urinalysis. Examination by a veterinary ophthalmologist was done prior to start and again during Week 13 of the study. At necropsy, gross and histologic examinations of various organs were conducted.

No clinical signs of toxicity, body weight changes, diagnostic abnormalities nor gross or microscopic lesions were noted at any dosage level. Ophthalmoscopic examination revealed "some increased granularity and mild hyper-reflectivity of the fundus" in the high-dosage group, possibly indicating a mild retinopathy. It was not considered toxicologically important. No cataracts were reported. A slight increase in hemosiderosis of the bone marrow in the high- dose group could not be properly assessed with the group size in this study. The small number of animals evaluated precludes the determination of a NOAEL or LOAEL for this study.

b. Twenty-Six-Week Study

Levine et al. (1983) studied the effects of TNT (approximately 99% pure) administered daily, by means of a gelatin capsule containing TNT mixed with Purina Certified Rodent Chow to reduce the hazards of explosion, to groups of six beagle dogs per sex at 0.0, 0.5, 2, 8, or 32 mg/kg/day for 26 weeks. (Study report indicates that dogs were administered TNT capsules for 25 weeks with a blank capsule administered daily for one week prior to TNT administration.) Animals were approximately six and one-half months old at the start of the TNT dosing schedule and were maintained throughout on daily rations of Purina Dog Chow. Animals were observed several times daily, before and after dosing, for toxic signs and were examined weekly by palpation for detectable masses. Body weight and food intakes were recorded weekly. Other toxicologic endpoints included a comprehensive clinical chemistry and hematological evaluation, urinalyses, and periodic electrocardiography (ECG) and ophthalmic examinations. During Week 27, all animals, following a 16-18 hour fast, were sacrificed by injection of intravenous pentobarbital sodium; major organs were weighed and all organs were collected and fixed for microscopic examination. Statistical analyses were performed.

At the highest dose tested (32 mg/kg/day), TNT was found to be lethal, with one female sacrificed in a moribund condition during Test Week 14 and another found dead during Test Week 16. Clinical signs in these two animals included dehydration, emaciation, jaundice, hypothermia, weight loss, diarrhea and ataxia. Clinical signs of toxicity observed in dogs surviving this lethal dose level included orange-brown urine and feces (also observed, to a lesser extent, at 8 mg/kg/day), darkening of the tongue and/or gums, jaundice and ataxia. In addition, body weights were reduced in all TNT-treated dogs with significant losses evident at 8 mg/kg/day (males only) and 32 mg/kg/day. High-dosed dogs also showed significantly reduced food intake throughout most of the study with similar losses evident at the 8 mg/kg/day level during Test Week 1. Urine was a light to dark brown color in dogs at the 32 mg/kg/day and, to a significantly lesser extent, at the 8 mg/kg/day level throughout the study. Additionally, during the final test week, urinary protein levels were increased in the two highest dose levels.

At the 32 mg/kg/day dose level, the observed jaundice was accompanied by elevated bilirubin levels in serum and urine and trace levels of urobilinogen. These findings were consistent with the observed anemic state (as evidenced by significant reductions in Hct, Hgb, and RBCs) for both sexes receiving either 8 or 32 mg/kg/day. Compensatory responses to anemia included increased numbers of reticulocytes, macrocytosis, and elevated numbers of nucleated RBCs. Leukocytosis with neutrophilia was evident in a dose-related manner at the two highest doses along with methemoglobinemia. These observations, along with evidence of a hemosiderin-like pigment in macrophages of the spleen and liver and sinusoidal congestion of the splenic red pulp with accompanying increased spleen size, suggested to the authors that TNT-induced anemia was hemolytic in origin. Reduced numbers of erythrocytes and their precursors in bone marrow were also seen.

Other abnormalities in blood chemistry parameters included statistically significant increases in serum globulin and LDH, dose dependent decreases in SGPT, with females showing significant reductions down to the 2 mg/kg/day level, and decreased glucose at 32 and 8 mg/kg/day (males only). Cholesterol levels were variable with slight increases at 8 mg/kg/day (males only) and slight decreases at 32 mg/kg/day (females only).

No definitive treatment-related effect was reported upon ophthalmic examination although some vitreal stranding or haze was observed at all levels (controls, 17%) with the two highest levels showing a somewhat higher incidence (42% to 50%). No ocular lesions were observed. Likewise, electrocardiography tracings did not reveal any TNT-related effects.

Several indications of liver injury were observed upon gross and histologic examination. Male (8 and 32 mg/kg/day) and female (32 mg/kg/day) dogs had significant increases in relative and/or absolute liver weight accompanied by moderate to marked hepatocytic cloudy swelling and hepatocytomegalia seen at the high dose and, to a lesser degree of severity, at all dose levels with lesions at the low dose (0.5 mg/kg/day) described as trace to mild. No such lesions were seen in the control animals. Microscopic evidence of cirrhosis was seen, primarily in males, at the 8 and 32 mg/kg/day dose levels and hemosiderosis of the liver was seen in the majority of dogs at the two highest levels as well as one female at the 2 mg/kg/day level. None of these microscopic lesions were seen in the two females necropsied prior to termination of this study.

The absolute and relative weight of the spleen of both sexes was significantly increased at the high-dose level with a significant increase in the relative weight in females at the 8 mg/kg/day level. This finding corresponded to a marked to severe generalized congestion, primarily at the two high-dose levels. Hemosiderosis of the spleen was evident at all dose levels and extramedullary erythropoiesis was demonstrated primarily in the high-dose group.

Absolute heart weights were significantly decreased in males at the two highest dose levels but no corresponding pathology was reported. Increases occurred in the absolute weight of the thyroid of females at the high dose and was accompanied by bilateral C-cell hyperplasia in all groups, including controls, but with a greater degree of severity in the high-dose group. The testes of male dogs receiving any level of TNT were unaffected.

Other notable pathological findings apparently related to TNT intake included membranous enteritis of the small intestine and erythroid hypoplasia at all TNT-treatment levels of both sexes, possibly due to administration of the TNT as a bolus dose. Enlarged, pigmented lymph nodes with no apparent histopathology were seen, primarily in the high-dose females. The 0.5 mg/kg/day test level appears to be a LOAEL for liver effects with histopathology at this level indicated as trace to mild. No effects were seen on the liver enzymes and organ weight at this low dose level nor on the spleen, another consistent target organ for toxicity. Therefore, a LOAEL of 0.5 mg/kg/day is considered to be appropriate.

c. Lifetime Exposure

Data on the toxic effects of lifetime exposure to TNT are available in an extensive series of studies by Furedi et al. using Fischer 344 rats (1984a,b,c) and B6C3F1 hybrid mice (1984d,e,f).

In the rat study, groups of 75 animals per sex (approximately 6-7 weeks old) received TNT (~ 99% pure) mixed in a diet of Purina rodent chow meal at dose levels of 0.0, 0.4, 2, 10, or 50 mg/kg/day for 24 months. Diets were prepared weekly, by sex, on the basis of projected body weight and intake data. All animals were observed daily for signs of toxicity; examination by palpation, and body weight and food intake determinations were evaluated weekly through Week 13 and bi-weekly thereafter. Ten rats/sex/dose were sacrificed at 6 and 12 months of treatment with the remaining rats sacrificed during Weeks 105-106. Blood was collected via the orbital sinus; animals were fasted for 17-19 hours and weighed prior to their sacrifice by carbon dioxide euthanasia. Major organs were weighed, and all tissues were fixed for histological examination. Clinical evaluations included hematology, clinical chemistry, ophthalmology, and gross and tissue morphology; statistical analyses were performed.

The chronic administration of TNT at doses up to 50 mg/kg/day did not alter mean survival times. Dose-related reductions in food consumption and a corresponding decrease in body weight gain were seen in both sexes at 10 and 50 mg/kg/day. At the high-dose level, these effects were statistically significant in the first few weeks of the study and remained so throughout. At 10 mg/kg/day, the changes developed somewhat later and tended to be more sporadic.

Anemia evidenced by a reduced Hct, Hgb and RBC count was seen at 10 and 50 mg/kg/day. The parameters were generally significant in both sexes receiving the high dose, beginning at Week 14 and continuing through termination. At the 10 mg/kg/day level, these same parameters were significant in males through Week 52 but were inconsistent in females. Methemoglobin values and/or percent methemoglobin were significantly increased in males receiving the high dose through their terminal sacrifice and in high-dose females at the Week 78 analysis. At 10 mg/kg/day, these parameters were significant in males only and only through the first year. Beginning at Week 52, a sporadic increase in platelets, lymphocytes and/or WBC was significant in the high-dose females, with an occasionally significant increase in all or some of these parameters in the high-dose males. In general, male rats appeared to be somewhat more sensitive than females to the hematological effects of TNT. An increased production of reticulocytes was seen as a compensatory response to the anemic state. Related lesions detected at terminal sacrifice and/or in rats found dead or sacrificed moribund included focal to multifocal myelofibrosis of the bone marrow, mild to moderate in severity, and significantly increased in incidence in the females receiving TNT at levels of 2.0 mg/kg/day and above (See Incidence Table A2-1 in Appendix 2.). At the high-dose level, this lesion was seen in 17/54 females (31.5%; $p>0.01$) but was not seen in males. Splenic lesions consisting of sinusoidal congestion, extramedullary hematopoiesis, and increased quantities of a hemosiderin-like pigment were seen in both sexes, generally at the two highest dose levels, with the incidence and severity of the diffuse sinusoidal congestion also significantly increased at the 2.0 mg/kg/day dose level in females. These lesions in the spleen were first detected during the six-month scheduled sacrifice and remained significant through termination. The weight of the spleen was significantly increased in males and females through most of the study (not significant in females at Week 104) at the high-dose level and was sporadically increased in both sexes at 10 mg/kg/day. The authors suggested that TNT appeared to induce anemia by a hemolytic process. This suggestion was further supported by the observance, minimal in nature, of Howell-Jolly and Heinz bodies at the 50 mg/kg/day level and the presence of methemoglobin in the circulating blood, suggesting the oxidizing nature of TNT and/or its metabolities.

Liver injury at 50 and, to a lesser extent, at 10 mg/kg/day was indicated upon gross examination by focal and multifocal red to tan areas. Increases in absolute and relative liver weight were evident in both sexes at the two highest dose levels. Upon histological examination, a dose-related increase in the incidence of foci and areas of hepatocellular hyperplasia with cystic degeneration was observed for males but not females receiving the 10 and 50 mg/kg/day dose during the second year of the study. Hepatotoxicity was also suggested by altered lipid and protein metabolism as evidenced by increased serum cholesterol, total protein and albumin levels and a more sporadic increase in globulin levels. Albumin/globulin ratios were significantly decreased. These chemical alterations were most predominant at the high-dose level but cholesterol levels in males were also significantly increased at the 2.0 and 10.0 mg/kg/day levels.

Blood urea nitrogen was slightly elevated at 50 mg/kg/day, particularly among females. Renal injury was indicated grossly as spotted, granular and cystic kidneys. Histological examination revealed a dose-related increase in incidence and severity of mild to moderate pigmentation, beginning at the 2.0 mg/kg/day dose level in females and the 10 mg/kg/day level in males. Inflammation with lymphocytic infiltration was apparent in both sexes at the high-dose level and in females at the 10 mg/kg/day level. Hyperplasia of the renal pelvis was also significantly increased in the females receiving the 50 mg/kg/day dose level. Kidney weights were elevated for animals of both sexes receiving 10 or 50 mg/kg/day. The brown mottled kidneys seen at necropsy for high-dose animals contained iron-negative cytoplasmic bodies and, nuclear hypertrophy of cortical proximal convoluted tubular cells was observed microscopically in males at 2 mg/kg/day or greater. Additional toxic effects on the urogenital system, primarily seen in high-dose females, included urinary bladder hyperplasia, papilloma, and carcinoma (discussed in section 5. Carcinogenicity).

No effects, other than a significant increases in absolute weight, were seen on the testes of males receiving the two high-dose levels. No other effects related to TNT intake were reported.

Except for an increased frequency of ocular discharge in the high-dose rats, no treatment-related abnormalities were detected either by ophthalmic examination or histological evaluation. A number of lesions were detected at all dose levels, with increased frequency versus time, but were considered to be related to ocular trauma or penetration at the time of orbital bleeding. An apparent NOAEL of 0.4 mg/kg/day is based on the absence of effects of TNT on the spleen, kidney, and bone marrow.

In the 24-month study conducted by Furedi et al. (1984d,e,f) in B6C3F1 hybrid mice, TNT (>99% pure) was administered in a diet of ground Purina chow to groups of 75 mice/sex/group at dosage levels of 0.0, 1.5, 10, or 70 mg/kg/day. Animals were observed daily for signs of toxicity. Examination by palpation, measurement for weight changes and food intakes and examination of bedding for red staining were conducted weekly through Week 13 and bi-weekly thereafter. Periodic ophthalmic examination as well as measurement of hematological and clinical chemisty parameters were conducted at defined intervals throughout the study. Ten mice/sex/dose were killed at 6 and 12 months with surviving animals killed after 24 months of treatment. Euthanasia was accomplished by carbon dioxide anesthesia following a 2 to 5 hour fast. Major organs were weighed, and all organs were fixed for histological evaluation. Tissues from control and high-dose mice underwent a comprehensive examination with those of the remaining levels undergoing a more limited examination. Appropriate statistical analyses were conducted.

The TNT did not cause deaths at the doses tested in this study; mean survival times were similar among control and treatment groups. Clinical signs related to TNT administration were not readily apparent. Reductions in body weight gains at the 70 mg/kg/day dose level were approximately 10% for both sexes up through the first 6 to 8 months of the study, with further reductions of about 15% for females and 20% for males through the remaining test period. An approximate 5% to 7% reduction in body weight gain for males but not females at the 10 mg/kg/day dose level for the majority of the study was not statistically significant. Body weights were not affected in mice given 1.5 mg/kg/day. Food intakes were variable with significant decreases apparent in high-dose males through approximately Week 19 and significant increases over control intakes beginning at Week 25. In females receiving the high dose, dietary intakes were significantly increased through most of the study, with a significant decrease in intake only during Week 1. At other dose levels, significant differences were sporadic in nature and varied from increases to decreases throughout.

Hematologic observations included anemia in both sexes administered TNT at 70 mg/kg/day, as evidenced by generally significant reductions in Hct, Hgb and RBC counts from Test Week 27 through Test Week 79. The effect was mild, was no longer apparent in males and was apparent but not significant in females by Test Week 105. Normal physiologic compensatory responses to the anemic state (e.g. reticulocytosis, macrocytosis, etc.) were not apparent. Lymphocytes and

WBC's were significantly increased during Test Weeks 27 and 52. Males receiving 10 mg/kg/day also had increased lymphocyte counts at Test Week 27. No consistently significant changes in clinical chemistry parameters were evident in this study.

Occasional elevations in relative liver, kidneys, spleen and heart weights were seen in mice receiving 70 mg/kg/day. Although statistically significant, these changes were small, and no pattern with respect to sex or time was observed, nor were absolute organ weights significantly different from controls.

Ophthalmic abnormalities were random and not considered to be related to treatment with TNT. A high incidence of cataracts in all test groups was significant in low-dosed females only, was not dose-related and was considered "spurious" and related to aging changes.

No TNT-induced gross lesions nor microscopic abnormalities were observed at the 6 and 12 month interim sacrifices. Increased extramedullary hematopoiesis of the spleen, cytoplasmic vacuolization of renal tubules (males), renal lymphocytosis (females) and a variety of inflammatory dermal lesions were considered spontaneous and were observed in control and treated animals alike. Enlargement of the spleen and lymph nodes in females receiving 70 mg/kg/day was observed at the 24 month necropsy as well as in mice that died or were sacrificed moribund between 12 and 24 months. The study authors reported that the incidence of combined leukemia/malignant lymphoma in the spleen of females increased with dose. They reported that the increase was statistically significant ($p<0.5$) at the 70 mg/kg/day dose level (high dose) and that the lesions were considered to be treatment-related. This was an inappropriate conclusion based upon current NTP guidelines (McConnell et al., 1986). These guidelines indicate that it is appropriate to combine all types of malignant lymphoma and lymphocytic leukemia, but not in a single organ. These types of tumors occur throughout the hematopoietic system. Upon recounting these tumors, by each sex or both sexes combined, in the whole animal, the statistical significance is lost (i.e., $p>0.05$) using the Fisher-Irwin Exact Test to compare dosage groups and the Cochran-Armitage Test for Trend. Therefore, based upon the statistical analyses, this study is considered to be negative with no tumors related to TNT exposure. The NOEAL for this study, based on the absence of body weight reduction and other effects, was 1.5 mg/kg/day.

3. Reproductive Effects

No data were available in the literature concerning the reproductive effects of TNT. It should be noted, however, that rats exposed to TNT in the diet at levels ranging from 25 to 300 mg/kg/day for periods of 4 to 13 weeks showed varying degrees of testicular atrophy and hyperplasia as well as other testicular effects. (These studies are fully described in Section VI.B. -

Short-term Exposure, Four-week Studies and Longer-Term Exposure, Thirteen-week Studies.) These effects were not evident in the Lifetime Exposure studies; nor is the significance, if any, of these effects on the reproductive capacity of the rat known.

4. Developmental Effects

No data were available in the literature concerning the developmental effects of TNT.

5. Carcinogenicity

The carcinogenic potential of TNT was evaluated in 24-month studies in Fischer 344 rats (Furedi et al., 1984a,b,c) and in hybrid B6C3F1 mice (Furedi et al., 1984 d,e,f).

In the study in rats, TNT was administered at 0.0, 0.4, 2, 10, or 50 mg/kg/day by diet to dosage groups of 75 rats per sex. Histopathologic lesions observed in females dosed at 10 and 50 mg/kg/day during the 12 to 24 month TNT treatment period included an increase in the incidence and severity of hyperplastic, preneoplastic and neoplastic lesions of the mucosal epithelium of the urinary bladder. Malignant neoplastic changes as well as benign neoplastic changes were present in the bladder epithelium. Based on these observed changes, the authors considered TNT a carcinogen to F344 rats under conditions of the study. The incidence of urinary bladder hyperplasia among females rats receiving the high dose was 12/55 (21.8%; $p<0.01$), for urinary bladder papilloma, 5/55 (9.1%; $p<0.05$) and for urinary bladder carcinomas, 12/55 (21.8%; $p<0.01$) (See Incidence Table A1-1 in Appendix 1.); metastases to other tissues were not observed. Bladder papilloma and carcinoma were not observed in the controls nor were these tumors observed in males.

In the mouse study, TNT was administered in the diet for up to 24 months. Groups of 75 mice per sex received TNT at doses of 0, 1.5, 10, or 70 mg/kg/day. Ten mice per sex per dose were killed following 6 and 12 months on test with surviving animals killed after 24 months of treatment. The major systemic effects observed in the high (70 mg/kg/day) dose group included anemia with hepatotoxicity. This indicates that the MTD was achieved. The study authors reported that the incidence of combined leukemia/malignant lymphoma in the spleen of females increased with dose. They reported that the increase was statistically significant ($p<0.05$) at the 70 mg/kg/day dose level (high dose) and that the lesions were considered to be treatment-related. This was an inappropriate conclusion based upon current NTP guidelines (McConnell et al., 1986). These guidelines indicate that it is appropriate to combine all types of malignant lymphoma and lymphocytic leukemia, but not in a single organ. These types of tumors occur throughout the hematopoietic system. Upon recounting these tumors, by each sex or both sexes combined, in the whole animal, the statistical significance is lost (i.e., $p>0.05$) using the Fisher-Irwin Exact Test to compare dosage groups and the Cochran-Armitage Test for Trend. Therefore, based upon the statistical analyses, this study is considered to be negative with no tumors related to TNT exposure.

6. Genotoxicity

The strong mutagenic activity of TNT was reported by Ellis et al. (1978). As little as 10 µg/plate of TNT, dissolved in dimethylsulfoxide (DMSO), with or without metabolic activation, was mutagenic in *Salmonella typhimurium* strains TA-98, TA-1538, and TA-1537, indicators of frame-shift reverse mutations; at 30 µg/plate with or without metabolic activation, mutagenic effects were noted in TA-100 as well as in those three strains. At 300 µg/plate with metabolic activation, TNT was positive in all five tester strains including TA-1535, indicating that TNT is positive for both frame-shift reverse mutations and base-pair substitutions.

Simmon et al. (1977) conducted studies using bacteria (*S. typhimurium*, strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100), with and without S9 metabolic activation, and yeast (*S. cerevisiae*) to evaluate the mutagenic activity of TNT before and after application of chlorination or ozonation disinfection techniques. Results were negative both before and after either disinfection technique except for two experiments in which TNT was reported as appearing weakly mutagenic (<2-fold increase in revertants) in TA-100 without metabolic activation. Using the same standard for mutagenicity as in the study by Ellis et al. (1978), i.e. a mutagenic ratio[1/] ≥ 2.0 as positive, none of the concentrations tested in the Simmon study would be positive. It is important to note, however, that the concentrations tested in these experiments were generally below 10 µg/plate with 33.5 µg/plate being the highest concentration tested in a single experiment. The increases seen in this study, while generally occurring at the highest concentration, were not always "dose-related".

Dilley et al. (1978) reported that TNT dissolved in DMSO and incubated at 10 to 500 µg/plate increased reverse mutation rates in a dose-related manner in *S. typhimurium* strains TA-1537, TA-1538, TA-98, and TA-100, both in the presence and absence of the S-9 metabolic activation system. The toxicity and mutagenicity of TNT were reduced upon metabolic activation.

Dilley et al. (1978) performed *in vivo* cytogenetic analyses on bone marrow cells from two groups of five young male Sprague-Dawley rats, each treated for 28 days with TNT at 0.25% (190.4 mg/kg/day) or 0.002% (1.8 mg/kg/day) in the

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1/ number revertants in test/number revertants in control feed and from two additional groups of five rats, each similarly treated and allowed to recover for 28 days. The evaluation procedure used was a modification of the method outlined by Nichols et al. (1972) in which the treated rats were injected, one and one-half hour prior to sacrifice, with 0.75 mg/kg of colchicine to obtain a high number of cells in metaphase. The bone marrow cells were obtained by aspiration from the distal end of the femur. Although the high dose was considered the highest tolerable dose, no rats died prematurely. Mitotic indices were depressed in the rats exposed to the high dose, but no cytogenetic abnormalities were observed. Following 28 days for recovery, the proliferative capacity of the bone marrow cells returned to normal levels as indicated by the mitotic indices. The authors concluded that, based on their assay, genetic damage induced by TNT was not evident.

In vitro measurement of unscheduled DNA synthesis (UDS) in human diploid fibroblasts (WI-38 cells) was also conducted by Dilley et al. (1978) in the presence and absence of a metabolically active system obtained from the liver of adult male mice. A dilution of TNT in DMSO was added to the culture medium to yield concentrations ranging from 0 to 1000 μg/ml. Results in this system, without metabolic activation, indicated that UDS was suggested at the higher concentrations. A definitive evaluation, however, was obscured by discoloration of the samples at the two highest concentrations, 500 and 1000 μg/ml, thus interfering with the colorimetric determination of DNA content. In the presence of metabolic activation, at concentrations ranging from 0 to 6000 μg/ml, UDS was not observed, however, the solubility of TNT was a limiting factor in this test (a precipitate was observed at all concentrations of TNT).

Ashby et al. (1985) reported that TNT gave a negative response in a mouse bone marrow micronucleus assay in which the mice were administered TNT by intraperitoneal injection at levels up to 80 mg/kg (described as 80% of the Maximum Tolerated Dose, MTD) and evaluated at 24, 48 and 72 hours after dosing for an increase in the presence of micronuclei.

In an in vivo/in vitro rat liver assay for UDS, conducted by Ashby et al. (1985), the hepatocytes of TNT treated rats were evaluated in an in vitro system following administration of TNT to the intact animal at dose levels up to 1000 mg/kg. A negative response was also observed in this system, which is used to measure genotoxic-carcinogenic response.

7. Other Effects

Male and female adult Sprague-Dawley rats were fed TNT in the diet at 0.25% for three weeks (Dilley et al., 1978) for the purpose of evaluating the ability of TNT to stimulate the hepatic microsomal enzyme system. Animals were killed by decapitation, and the livers were removed and prepared for analysis by standard methods. Substrates used represented three metabolic pathways: N-demethylation, O-demethylation and aromatic ring hydroxylation. Results indicated that TNT showed no stimulatory activities in two of the three systems, with a limited positive response in the O-demethylation system as indicated by a stimulation of the metabolism of o-nitroanisole. However, metabolism of TNT itself is not altered by pre-treatment of the rat with phenobarbital, TNT or RDX, indicating to the authors that the decreased toxicological manifestations to repeated dosing of TNT cannot be explained on the basis of an increased metabolic disposition of TNT.

VII. HEALTH ADVISORY DEVELOPMENT

Available toxicity studies in various animal species, for periods ranging from a single oral dose (LD_{50}) to continuous 24-month feeding studies, along with data on the health effects in humans exposed to TNT in the atmosphere have been evaluated. Several toxicity endpoints considered relevant to a HA for TNT in drinking water have been identified. Numerous signs and symptoms of TNT toxicity in humans exposed in the workplace have been reported (Zakhari and Villaume, 1978), to include such relatively mild effects as respiratory irritation, skin lesions, and gastrointestinal disorders and progressing to more severe symptoms such as methemoglobinemia, jaundice, aplastic anemia, cataract formation, menstrual disorders, neurologic dysfunction and nephrotoxicity. Of these disorders, the most consistently reported effects of TNT exposure in humans, including those which have been reported as the principal cause of death when such exposure resulted in mortality, are hepatitis and aplastic anemia (Zakhari and Villaume, 1978).

Human exposure data gathered through occupational health surveys conducted at various Army Ammunition Plants have indicated that atmospheric exposure to TNT at levels ranging from <0.02 to >3.0 mg/m^3 for periods generally up to 6 months consistently caused abnormalities in Hgb, Hct, and RBC count (estimated absorbed dose could not be determined from the available data). Abnormalities in other hematological parameters and such clinical chemistry parameters as BUN, SGOT, LDH and bilirubin have also been reported (Friedlander et al., 1974; Morton and Ranadive, 1974; Buck and Wilson, 1975). In almost all cases, removal of the affected individuals from the source of exposure has resulted in a return of these parameters to normal levels, although the time required for recovery could not be determined. A consistent sign of TNT exposure has been a red discoloration of the urine, apparently due to unidentified TNT metabolites.

In animals, significant and consistent findings following feeding of TNT in the diet include hemolytic anemia with compensatory responses such as reticulocytosis and macrocytosis; methemoglobinemia; increased spleen weight usually associated with hemosiderosis and, in lifetime studies, congestion and extramedullary hematopoiesis; and an increase in liver weight generally associated with increased cholesterol, decreased SGPT, pigmentation, hyperplasia and hepatocytomegaly (Dilley et al., 1978, 1982; Levine et al., 1981, 1983, 1984a,b; Furedi et al., 1984a-f). Reported effects of TNT on the testes (atrophy and hyperplasia) have been less consistent as have effects on other hematological and clinical chemistry parameters. Consistent decreases in body weight gain at high dose levels seem to be associated with a corresponding decrease in food intake. Red color appeared in the urine of rats, mice and dogs fed TNT in the diet at levels of approximately 5 mg/kg/day or greater, depending upon species.

Anemia in dogs, rats and mice fed TNT, evidenced by decreases in Hgb, Hct and

RBC count, first appeared in dogs after four weeks of feeding at 20 mg/kg/day (Dilley et al., 1978, 1982) and remained evident when TNT was fed at doses of 8 mg/kg/day or more for up to 26 weeks (Levine et al., 1983). Hematological signs of anemia also appeared in rats when TNT was fed at doses ranging from 25 to 300 mg/kg/day for periods of 4 to 13 weeks (Dilley et al., 1978, 1982; Levine et al., 1981, 1984b). In a two-year rat study (Furedi et al., 1984a), the anemia was associated with a statistically significant increase in focal to multifocal myelofibrosis of the bone marrow along with an enlarged spleen and splenic lesions consisting of sinusoidal congestion, extramedullary hematopoiesis and hemosiderosis. These effects were significant in females at 2.0 mg/kg/day and above and in males at 10 and 50 mg/kg/day. Mice were less sensitive to the effects of TNT on the hematological parameters for anemia with only slight or no effects at doses up to 700 mg/kg/day for periods up to 13 weeks (Dilley et al., 1978, 1982; Levine et al., 1984a). When mice were fed diets containing 70 mg/kg/day TNT for up to two years, anemia was evidenced by decreases in the Hgb, Hct, and RBC counts by Week 79, but these changes were evident but no longer significant by Week 105 (Furedi et al., 1984d).

The hepatomegaly reported in dogs and rats fed TNT for periods up to 13 weeks was generally not associated with histological abnormalities except for a multifocal or diffuse hepatocellular hypertrophy (hepatocytomegaly) in two groups of rats fed TNT at 125 or 300 mg/kg/day for 13 weeks (Levine et al., 1981, 1984b). When TNT was fed to dogs at lower levels (0.5, 2, 8, or 32 mg/kg/day) for a longer duration (26 weeks), the hepatomegaly was accompanied by moderate to marked hepatocytic cloudy swelling and hepatocytomegalia at all treatment levels, but of only trace to mild severity at the lowest dose (Levine et al., 1983). In the two-year feeding study in rats (Furedi et al., 1984a,c), the liver injury was further evidenced by a dose-related increase in the incidence of foci and areas of hepatocellular hyperplasia with cystic degeneration in the males, but not females, fed TNT at 10 and 50 mg/kg/day. Clinical chemistry parameters, while not always significant, were generally indicative of altered liver function as indicated by increased cholesterol, total protein, albumin and globulin levels. These alterations in liver morphology were not evident in the mouse (Furedi et al., 1984d,f).

Testicular atrophy and hyperplasia, seen in high-dose rats (160 mg/kg/day) receiving TNT in the diet for up to 13 weeks, were first detected in those animals sacrificed after four weeks of treatment (Dilley et al., 1978, 1982). Similar dose related effects were seen in rats fed TNT at doses of 25, 125 or 300 mg/kg/day for 13 weeks (Levine et al., 1981, 1984b). The testicular atrophy in this study was first noted by palpation in the highest-dosed group during Week 6. No testicular effects were evident at 5 mg/kg/day. Similar testicular effects were not evident in rats fed TNT for up to two years. In contrast, significant findings in the two-year study (Furedi et al., 1984a,c) indicated an increase in the absolute weight of the testes at the two highest dose levels (10 and 50 mg/kg/day) with no treatment-related histopathology.

Female rats fed TNT at levels up to 50 mg/kg/day for two years (Furedi et al., 1984a,c) showed a significant increase in incidence and severity of hyperplastic, preneoplastic and neoplastic (papilloma and carcinoma) lesions of the mucosal epithelium of the urinary bladder at the 50 mg/kg/day dose level (Table A1-1, Appendix 1). This carcinogenic effect was not evident in males and was not reported in any other species.

While the development of cataracts has been reported in humans exposed chronically to TNT (Zakhari and Villaume, 1978; Hassman, 1968; Harkonen et al., 1983), no such lesions were found in rats and mice exposed to TNT at doses up to 50 and 70 mg/kg/day, respectively, for up to two years and periodically subjected to a comprehensive ophthalmological examination throughout the two-year study (Furedi et al., 1984b,e). No lesions or cataracts were found in dogs fed TNT at levels up to 32 mg/kg/day for 26 weeks (Levine et al., 1983) nor in dogs and monkeys treated with TNT at doses up to 1 mg/kg/day for 90 days (Hart, 1974; Martin, 1974). Cataract development observed in some human epidemiological studies does not provide sufficient data to estimate a health advisory.

Based on the foregoing data, the dog appears to be the species most sensitive to the toxic effects of TNT in the diet/bolus dose. In this species, systemic effects were associated with the liver, the organ system most commonly linked with TNT toxicity, and were apparent at a level of 0.5 mg/kg/day as evidenced by hepatomegaly with hepatocytic cloudy swelling and hepatocytomegalia of trace to mild severity (Levine et al., 1983). The dog also appears similar to man in its metabolism of TNT, with differences being largely quantitative in nature, and is considered the most appropriate animal model for estimation of a human health advisory.

A. Quantification of Toxicological Effects

Health Advisories are generally determined for One-day, Ten-day, Longer-term (approximately 7 years) and Lifetime exposures if adequate data are available that identify a sensitive noncarcinogenic end point of toxicity. The HAs for noncarcinogenic toxicants are derived using the following general formula:

$$HA = \frac{(\text{NOAEL or LOAEL}) \times (\text{BW})}{(\text{UF}) \times (\_\_\_ \text{ L/day})} = \_\_\_ \text{ mg/L } (\_\_\_ \text{ µg/L})$$

where:

NOAEL or LOAEL = No- or Lowest-Observed-Adverse-Effect Level (in mg/kg bw/day).

BW = assumed body weight of a child (10 kg) or an adult (70 kg).

UF = uncertainty factor in accordance with NAS/ODW guidelines.

_L/day = assumed daily water consumption of a child (1 L/day) or an adult (2 L/day).

1. One-Day Health Advisory

No data were located in the available literature that were considered suitable for the calculation of a One-day HA. Short-term studies were limited to assessments of acute oral LD_{50} values. It is suggested that the DWEL (20 ug/L) be used as a conservative estimate for the One-day HA.

2. Ten-Day Health Advisory

No appropriate data of less than 30 days duration were located in the available literature for the calculation of a Ten-day HA. The four-week studies of Dilley et al. (1978, 1982) in dogs, rats and mice evaluated only a small numbers of the available animals with the remaining animals being continued for a total of 13-weeks. A four-week range-finding study in mice (Levine et al., 1984a), the least sensitive species, used doses that increased by a factor of 7 and were not considered adequately sensitive for toxicological evaluation. It is, therefore, suggested that the DWEL (20 ug/L) be used as a conservative estimate for the Ten-day HA.

3. Longer-Term Health Advisory

Several studies were of appropriate duration to be considered for calculating a Longer-Term HA. Thirteen-week studies were conducted in dogs, rats and mice (Dilley et al., 1978, 1982; Levine et al., 1981, 1984b) and a 26-week study was conducted in dogs (Levine et al., 1983).

Mice were least sensitive to the toxic effects of TNT, showing only minimal but significant increases in spleen weight with hemosiderosis and slight but not significant decreases in Hct at the higher dose levels. No significant effects were evident on the liver. This species was not considered appropriate for HA development.

Dogs treated with TNT for 13-weeks at doses up to 20 mg/kg/day displayed evidence of anemia and liver toxicity indicated by changes in the clinical parameters. However, no clear-cut NOAEL could be determined as only two dogs/sex/level were available for analysis, with only one male surviving on the high-dose level.

In the 13-week study in rats, conducted by Dilley et al. (1978, 1982), anemia was evident at the three highest dose levels (7, 35 and 160 mg/kg/day) and was accompanied by significant effects on the spleen along with toxic effects on the liver, kidney and testes. However, several limitations in this study, including the small number of available animals, possible "systemic" errors in weighing procedures and a lack of histological data at the two lowest dose levels despite significant findings at the next higher level, precluded its use for calculating a Longer-term HA. The remaining 13-week study in rats (Levine et al., 1981, 1984b) was generally well conducted with good dose-response data but was considered to have too broad a dose range, with doses decreasing by a factor of five, to provide adequate evaluation of the lower levels. The lowest dose, 5 mg/kg/day, while apparently a NOAEL in this study, was higher than doses producing positive effects in other strains and species.

The 26-week study in dogs (Levine et al., 1983) remains as the most appropriate from which to derive a Longer-term HA. This study produced clearly toxic effects to the target organs for TNT, specifically the liver, spleen and hematopoietic system, at levels of 2 mg/kg/day and above, and trace to mild effects on the liver, described as hepatocytomegalia with hepatocytic clouding swelling, at the 0.5 mg/kg/day level (LOAEL). Based on NAS/ODW guidelines, use of a study of appropriate duration with a LOAEL in animals would require an uncertainty factor of 1000. Calculation of the Lifetime HA with these factors would produce a value equivalent to the calculated DWEL (Lifetime Health Advisory). It is, therefore, suggested that the DWEL (20 µg/L) be used as a conservative estimate for the Longer-term HA for both the 10 kg child and the 70 kg adult.

4. Lifetime Health Advisory

The Lifetime HA represents that portion of an individual's total exposure that is attributed to drinking water and is considered protective of noncarcinogenic adverse health effects over a lifetime exposure. The Lifetime HA is derived in a three step process. Step 1 determines the Reference Dose (RfD), formerly called the Acceptable Daily Intake (ADI). The RfD is an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious effects over a lifetime, and is derived from the NOAEL (or LOAEL), identified from a chronic (or subchronic) study, divided by an uncertainty factor(s). FROM THE RfD, A DRINKING WATER EQUIVALENT LEVEL (DWEL) CAN BE DETERMINED (STEP 2). A DWEL IS A MEDIUM-SPECIFIC (I.E., DRINKING WATER) LIFETIME EXPOSURE LEVEL, ASSUMING 100% EXPOSURE FROM THAT MEDIUM, AT WHICH ADVERSE, NONCARCINOGENIC HEALTH EFFECTS WOULD NOT BE EXPECTED TO OCCUR. The DWEL is derived from the multiplication of the RfD by the assumed body weight of an adult and divided by the assumed daily water consumption of an adult. The Lifetime HA is determined in Step 3 by factoring in other sources of exposure, the relative source contribution (RSC). THE RSC FROM DRINKING WATER IS BASED ON ACTUAL EXPOSURE DATA OR, IF DATA ARE NOT AVAILABLE, A VALUE OF 20% IS ASSUMED. If the contaminant is classified as a Group A or B carcinogen, according to the Agency's classification scheme of carcinogenic potential (U.S. EPA, 1986), then caution should be exercised in assessing the risks associated with lifetime exposure to this chemical.

The two-year studies in Fischer 344 rats and B6C3F1 mice (Furedi et al., 1984a-f) were well conducted lifetime exposure studies. The rat study clearly defined a NOAEL of 0.4 mg/kg/day for bone marrow, spleen, and kidney effects. At this dose level, there was no evidence of the splenic congestion, increased deposition of pigment in the kidneys or myelofibrosis of the bone marrow seen, primarily in females, at 2.0 mg/kg/day. Other effects seen at the higher doses (10 and 50 mg/kg/day) and generally in both sexes included anemia, increased pigmentation and extramedullary hematopoiesis of the spleen, inflammation of the kidney and hyperplasia of the renal pelvis, liver and urinary bladder. Urinary bladder papillomas and carcinomas were present at a significant level in the high-dosed (50 mg/kg/day) females.

Similar effects reported in the two-year studies conducted in mice included anemia and hepatomegaly without microscopic alterations at the high-dose level (70 mg/kg/day), and a dose-related lymphocytosis at the 10 and 70 mg/kg/day levels. The study authors reported that the incidence of combined leukemia/malignant lymphoma in the spleen of females increased with dose. They reported that the increase was statistically significant ($p<0.05$) at the 70 mg/kg/day dose level (high dose) and that the lesions were considered to be treatment-related. This was an inappropriate conclusion based upon current NTP guidelines (McConnell et al., 1986). These guidelines indicate that it is appropriate to combine all types of malignant lymphoma and lymphocytic leukemia, but not in a single organ. These types of tumors occur throughout the hematopoietic system. Upon recounting these tumors, by each sex or both sexes combined, in the whole animal, the statistical significance is lost (i.e., $p<0.05$) using the Fisher-Irwin Exact Test to compare dosage groups and the Cochran-Armitage Test for Trend. Therefore, based upon the statistical analyses, this study is considered to be negative with no tumors related to TNT exposure. The NOAEL for this study was 1.5 mg/kg/day.

The 26-week study in dogs (Levine et al., 1983) might also be considered for development of a Lifetime HA. This study resulted in clearly toxic effects to the target organs for TNT, specifically the liver, spleen and hematopoietic system, at levels of 2 mg/kg/day and above. At the 0.5 mg/kg/day level, this study produced hepatocytomegalia with hepatocytic cloudy swelling of trace to mild severity. No accompanying effects to the liver enzymes and organ weight were seen at this dose level. The 0.5 mg/kg/day level may, therefore, be considered a LOAEL for hepatic effects in the dog.

Based on the foregoing studies, it appears that the dog (Levine et al., 1983) is somewhat more sensitive to the hepatic effects of TNT than either rats or mice and may be considered the most appropriate species for calculating a Lifetime HA. When the study is viewed in relation to the two-year study in rats (Furedi et al., 1984a-c) with a NOAEL of 0.4 mg/kg/day, it is felt that the dose of 0.5 mg/kg/day in dogs is close to the actual threshold dose and that an uncertainty factor of 1000 will provide a sufficient margin of safety for humans.

TNT is classified EPA Group C, possible human carcinogen based on urinary bladder papilloma and carcinoma in female Fischer 344 rats.

Step 1: Determination of Reference Dose (RfD)

$$\text{RfD} = \frac{(0.5\ \text{mg/kg/day})}{(1000)} = 0.0005\ \text{mg/kg/day}\ (0.5\ \text{ug/kg/day})$$

Where:

0.5 mg/kg/day = LOAEL, based on trace to mild effects on the liver of dogs exposed to TNT in the diet for 26 weeks.

1000 = Uncertainty factor: It is based on the primary study of Levine et al., 1983 and the strong supporting study of Furedi et al., 1984a-c. The LOAEL of 0.5 mg/kg/day in the Levine et al., 1983 study appears to be close to the actual threshold dose for liver effects. It approximates the NOAEL of 0.4 mg/kg/day in the Furedi et al., 1984a-c study in rats. Reproductive toxicity data are not currently available.

Step 2: Determination of a Drinking Water Equivalent Level (DWEL)

$$\text{DWEL} = \frac{(0.0005\ \text{mg/kg/day})\ (70\ \text{kg})}{(2\ \text{L/day})} = 0.0175\ \text{mg/L}\ (\text{rounded to } 20\ \mu\text{g/L})$$

Where:

0.0005 mg/kg/day = RfD

70 kg = assumed body weight of an adult

2 L/day = assumed daily water consumption of an adult

Step 3: Determination of the Lifetime Health Advisory

The Lifetime HA is derived from the DWEL by factoring in other sources of exposure. The relative source contribution (RSC) is the percent of the total exposure to the chemical from drinking water. Actual data may be used when available. Hence, RSC may potentially vary from 1 to 100 percent. A value of 20% for RSC from drinking water is assumed in the absence of actual exposure data. Additionally, it is EPA policy that an additional uncertainty factor of be used for Group C carcinogens. A valid quantitative cancer risk assessment was developed from the Furedi et al. (1984a, c) study and is provided in Section VII B. This quantitative cancer risk assessment indicates that a minimal additional uncertainty factor is necessary to account for cancer risk. Hence, the additional uncertainty factor of 2 is applied to the Lifetime HA. For TNT the lifetime HA is as follows.

$$\text{Lifetime HA} = \frac{0.0175 \text{ mg/L } (0.2)}{2} = 0.00175 \text{ mg/L (rounded to 2 ug/L)}$$

Where:

0.0175 mg/kg/day = Drinking Water Equivalent Level (DWEL)

0.2 = Relative source contribution (RSC) assumption of 20%

2 = Uncertainty factor for Group C classification, ODW policy

B. Quantification of Carcinogenic Potential

TNT is classified EPA Group C based on urinary bladder papilloma and carcinoma that were observed in female Fischer 344 rats. Mutagenic activity was observed in the Ames test with and without metabolic activation. The risk manager must balance assessment of carcinogenic potential against the likelihood of occurrence of health effects related to noncarcinogenic endpoints of toxicity.

In order to assist the risk manager in this process, drinking water concentrations associated with cancer risks over the range of one excess tumor in populations of ten thousand(10^{-4}) to one excess tumor in populations of one million (10^{-6}) for the 70-kg adult, drinking 2 liters of water per day, are provided.

In the lifetime feeding study conducted by Furedi et al. (1984a,c), the high-dosed female rats exhibited a statistically significant ($p<0.01$) increase in the combined incidence of transitional cell papilloma and transitional cell carcinoma of the bladder. The combined incidence of bladder papilloma and carcinoma used to calculate the carcinogenic risk assessment are 0/54, 0/54, 0/55, 1/55 and 17/55 for female rats at doses of 0.0, 0.4, 2.0, 10.0 and 50.0

mg/kg/day, respectively. The multistage model was used for high-to-low dose extrapolation (Crump and Watson, 1979; Howe and Crump, 1982). Global83 was used to fit the data in the experimental dose range and to obtain upper 95% confidence-limits on the combined incidence of bladder papilloma and carcinoma. The multistage model conforms to a biological model of tumor initiation and promotion (Crump et al., 1977) and provided an adequate fit to the dose-response data for TNT. The relationship of the concentration (µg/L) of a chemical in drinking water to cancer risk is expressed as follows:

$$\frac{35000}{q_1^*} \times R = C$$

Where:

$q_1^* = (mg/kg/day)^{-1}$

R = risk (10^{-4}, 10^{-5}, 10^{-6}, etc.)

C = concentration of chemical in µg/L

35000 = conversion factor for mg to µg and assumption that a 70 kg adult drinks 2L of water/day

The animal doses were converted to equivalent human exposures using a surface area correction assuming a 0.30 kg rat, and a 70 kg human. The human slope factor (q_1^*) is 3×10^{-2} $(mg/kg/day)^{-1}$ for the linearized multistage model. The slope, q_1^*, is taken as an upper bound of potency of the chemical to induce cancer at low doses below the experimental dose range. Assuming that a 70 kg human adult consumes 2 liters of water a day over a 70 year lifespan the estimated cancer risk is as follows:

Level of Human Risk	Dose in µg/L
10^{-4}	100
10^{-5}	10
10^{-6}	1

For comparison purposes, drinking water concentrations associated with an excess cancer risk of 10^{-6} were 0.7 µg/L, 20 µg/L, 700 µg/L, 20 µg/L and 10 µg/L for the one-hit, multihit, probit, logit and Weibull models, respectively. The parameter estimates for these models were calculated with RISK81 (Kovar and Krewski, 1981).

The estimated excess cancer risk associated with lifetime exposure to drinking water containing TNT at 20 µg/L is approximately 2×10^{-5}. The estimated excess cancer risk associated with lifetime exposure to drinking water containing TNT at 2 µg/L is approximately 2×10^{-6}. This represents the upper 95% confidence limit on risk from extrapolation using the linearized multistage model. The actual risk is unlikely to exceed this value.

VIII. OTHER CRITERIA, GUIDANCE AND STANDARDS

The ACGIH (1986) 8-hour time-weighted average threshold limit value (TWA-TLV) for exposure to TNT is 0.5 mg/m^3. The 15-minute short-term exposure limit (STEL) has been eliminated pending additional toxicological data. The OSHA (1981) Permissible Exposure Limit (PEL) remains at 1.5 mg/m^3.

As summarized by NRC (1982), the following workroom standards have been adopted for TNT by various countries: Czechoslovakia, 0.1 ppm or 1 mg/m^3; West Germany, 0.15 ppm or 1.5 mg/m^3; East Germany, 0.15 ppm or 1.5 mg/m^3; and U.S.S.R., 0.1 ppm or 1 mg/m^3 (Verschueren, 1977). The U.S. Navy Bureau of Medicine and Surgery (BUMED, 1980) as cited in NRC (1982) has established a target interim maximum contaminant level (TIMCL) of 0.05 mg/L (50 µg/L) for TNT in drinking water. Dacre (1980) calculated an interim criteria for TNT for the protection of human health of 44.24 µg/L. Earlier, the U.S. Army had established limits of 1 mg/L (1000 µg/L) in drinking water and 5 mg/L (5000 µg/L) in water used by fish and wildlife (Smock et al., 1976, as cited in NRC, 1982). USAMBRDL (1980), as cited in NRC (1982), has recommended a TNT limit of 0.01 mg/L (10 µg/L) in wastewater, and the U.S.S.R. has set 1 mg/L (1000 µg/L) as the maximal permissible concentration in surface water (McKee and Wolf, 1963, as cited in NRC, 1982).

IX. ANALYTICAL METHODS

Several methods have been published for TNT analysis in water (TNT wastes). A simple, quick and reproducible method using high performance liquid chromatography (HPLC) is described.

The following methods for the determination of TNT in TNT wastes have been listed by Zakhari and Villaume (1978):

1. Colorimetric or spectrophotometric determination at 500 nm following treatment of wastes with sodium sulfite and sodium hydroxide solutions.

2. Colorimetric measurement of a Meisenheimer complex at 440 nm following treatment with 15% potassium hydroxide solution, usable to 80 ppm α-TNT (2,4,6-TNT).

3. Gas chromatographic detection of ppb to ppt in sea water using a nickel-63 electron capture detector.

4. Liquid chromatographic characterization after adsorption of nitro compounds on a styrene-divinylbenzene copolymer type resin.

The HPLC method described by Brueggeman (1983) utilizes a trace enrichment sample preparation technique to keep preparation time to a minimum and affords analysis to be conducted at ambient temperature without loss of efficiency of separation or speed of analysis. The detection limits were reported as 0.2 μg/ml. Several military explosives were analyzed by this method.

In the HPLC method, separation of the explosives is achieved by using a reverse phase column (C_{18}) and a mobile phase of methanol and water. A linear gradient elution program is used in which the eluent is changed from 95% Pump A (25% $MeOH/H_2O$) to 50% Pump B (80% $MeOH/H_2O$) in 30 minutes at a flow rate of 1.7 mL/min and a pressure of 500 psi. The column effluent is monitored at 240 nm. Separation was accomplished in <28 minutes.

Prior to analysis of the aqueous samples, the C_{18} cartridge is activated with two to four milliliters of methanol followed by 15 ml of glass-distilled water. This procedure removes organic impurities, displaces the methanol and activates the cartridge. Concentration of the sample is achieved by passing 20 ml of each sample, containing 200 ppm of the internal standard (1,3-dinitrobenzene), through the SEP-PAK cartridge at approximately 10 ml/min. followed by the sequential injection of 10 ml of air, 4 ml of a 50% acetonitrile/water solution and an additional 10 ml of air. The combined elements are collected in a screw-cap test tube and centrifuged at 2000 rpm for 15 minutes. The supernatant is analyzed by HPLC as described.

The ratio of peak area of TNT to peak area of the internal standard was plotted versus concentration of TNT. By examining the R^2 correlation coefficients, it was demonstrated that TNT showed a linear relation over the concentration range analyzed ($R^2 > 0.998$) over a 4-day period. The lower detection limit was 100 ng.

Recovery studies done with laboratory-spiked wastewater indicated a recovery range of 70% to 76% for TNT. The peak areas of the SEP-PAK-trapped materials were compared with standards having identical concentrations of the compound. Recovery of the internal standard over a 5-day period was considered reproducible (S.D.±2%). Separation of a synthetic wastewater influent and effluent showed good resolution for all compounds tested. This method, as described, is considered by the author to be suitably precise, accurate, sensitive, and selective for the determination of TNT and various other explosives in wastewater.

The analysis of actual wastewater samples were not reported by the Brueggmann procedure; however, Spanggord et al. (1978) reported on the analysis of several AAP wastewater samples (LAP discharges) using a similar HPLC procedure. Using a $C_{18-\mu}$ Bondpak Reverse-Phase column, a 60:40 methanol:water solvent at a flow rate of 1.6 ml/min. and uv detection at 254 nm, the retention time for TNT was 209 seconds in LAP discharge samples. Peak areas were determined by digital integration and quantitation was by the internal standard method using benzophenone. The lower limits of detection were 0.1 ppm and these limits could be lowered by a factor of two at 210 nm. This procedure did not utilize any special sample preparations or concentration techniques.

X. TREATMENT TECHNOLOGIES

Methods for wastewater treatment for the removal of TNT and its products are discussed. The current treatments of choice for both "red" and "pink" water are described in greater detail.

Waste water discharges from AAP's involved in the production of TNT are of two unique types:

1. "Red water" is the spent sellite waste solution formed during the TNT purification process. It contains TNT and its isomers, sodium carbonate, sodium sulfate, and sodium sulfite, along with complex chemicals resulting from the degradation of the TNT isomers. Many of the components of red water are toxic and/or carcinogenic and it has been classified as a "hazardous substance" by the EPA (Ryon et al., 1984).

2. "Pink water" is the aqueous effluent generated during TNT manufacture from plant clean-up and scrubbing processes, LAP operations and as a condensate from the evaporative concentration and incineration of red water. It contains varying amounts of TNT and its meta-isomers and photodegradation products of TNT to include water-soluble, organic-insoluble anions, organic solvent-extractable products and DNT isomers (Ryon et al., 1984).

Treatment methods for these two major types of TNT waste require different procedures which are treated separately below.

A. Red Water

Disposal of the wastes generated during the sellite purification process is the one of most serious concern. During the period between World War II and the Korean War, red water wastes were dumped into streams as a means of disposal. Later, after evaporative concentration of the red water at the AAP facility, the concentrates were either sold to paper mills for incineration and recovery of the sodium sulfate needed for the pulping process or incinerated at the AAP, followed by landfilling of the ash. Transportation of the concentrate is no longer feasible due to its hazardous classification and stricter pollution regulations have decreased the secondary market for the recoverable sodium sulfate. Incineration of the concentrate is not only expensive but, more importantly, adds to the existing air and solid waste pollution problem. Current techniques are aimed at the recovery of the sulfur and sodium present in the red water. Several companies and their processes are listed below:

1. Molten salt bath reduction process - Atomics International
2. Carbonate process - Tampella Smelt
3. Pyrolysis reduction process - SCA Billerud
4. Sulfite recovery process - SONOCO

Of these, the SONOCO process appears to be the most technically and economically feasible. Ryon et al. (1984) described the sulfite recovery process (SRP) as adapted for use at the Radford AAP as follows: red water is mixed with aluminum hydroxide and reduced in a furnace to sulfur dioxide and soluble sodium aluminate. The sulfur dioxide is scrubbed with sodium carbonate to form sodium sulfite, which is reused to purify TNT. The sodium aluminate is converted to aluminum hydroxide, which is recycled for the treatment of the red water feedstock. In summary, the Radford adaptation of the SRP, essentially a closed-loop process, converts red water to sellite which is reconverted to red water when used in the TNT purification process. Impurities from TNT itself are converted to water, nitrogen and carbon dioxide and released to the atmosphere. A more detailed description along with a flow diagram of this process is contained in Pal and Ryon (1986). Problems arising from this adaptation are currently under active investigation.

B. Pink Water

Under condition of full production of TNT, it has been estimated (Forsten, 1980 as cited in Ryon et al., 1984) that the amount of pink water generated could be as high as 100,000 gal/day/line and may contain 140-160 mg TNT/L along with other contaminants and explosives. The current method of choice for pink water abatement is adsorption by activated carbon (Ryon et al., 1984). While this method has been long and widely used, it is limited by a 24% drop in efficiency of the carbon with the first regeneration with successively lower losses at each subsequent regeneration (Castorina, 1980). To increase the efficiency and reduce the cost of carbon regeneration, a new thermal carbon regeneration process using a rotary kiln has been developed and put into use at the Iowa AAP. Ryon et al. (1984) described the process as follows: the explosive-laden carbon is dewatered and calcined at 110°C. In the second step, spent carbon is pyrolyzed at 300°C to remove the adsorbed explosives. Steam at 20 kg/hr and carbon dioxide at 25 L/min are fed at this stage to maintain a reducing atmosphere. In the third step, the carbon is subjected to a reactivation temperature of 861°C. An average regeneration efficiency of 92%, decreasing significantly with time, has been achieved (Forsten, 1980 as cited in Ryon et al., 1984). The carbon can be regenerated four times using this process after which it is discarded by open burning (Pal and Ryon, 1986).

A study conducted at the Holston AAP (Burrows, 1982) indicated that while adsorption on granular activated carbon (GAC) is a viable treatment method for removal of TNT from deionized water when present alone, this would not be a method of choice for mixtures of munition compounds as competition for adsorption sites and reduced overall efficiency has been demonstrated. Other munitions are progressively displaced in favor of TNT adsorption.

A hydroperm microfiltration system has been proposed as an alternate means of treating pink water (Sundaram et al., 1981 as cited in Pal and Ryon, 1986).

This method is based on cross-flow filtration with thick-walled, porous plastic tubes (hydroperm tubes) which have been demonstrated to remove significant levels of high-molecular-weight dissolved solids and, in the case of pink water, the color associated with these constituents. The effluent from the system can be recycled in LAP operations or discharged into natural streams after treatment with carbon. The feasibility of this system is yet to be determined.

C. Other Methods

While open burning of waste munitions is still practiced at AAP's, incineration is becoming the preferred method. In the treatment of TNT wastes, open burning or incineration of the red water concentrate and the spent carbon from pink water treatment has been practiced at various AAP's. In many cases the incineration process, despite its potential for the release of new and unmonitored chemicals to the atmosphere, is preferable to storage in landfills or elsewhere. New hazardous waste disposal furnaces are being developed and tested at various facilities. Pal and Ryon (1986) have described the following:

1. Rotary Kiln Incinerator - propellant slurries are incinerated by a continuous process at 1000°C in refractory lined cylinders rotating at a slow speed. Capital and maintenance costs are high in this system.

2. Fluidized Bed Incinerator - incorporates a slurry feed system (for the aqueous explosive slurries), a cyclone particulate collector and a stack gas analyzer. The addition of 6% (by weight) of nickel oxide to the aluminum bed has produced a drastic reduction in the emissions making this method safe, efficient and economical.

3. Pyrolytic Incineration - a two step, continuous process system combining pyrolysis and combustion in a conventional incinerator in one step with a pyrotherm system (pyrolytic system with heat recovery) in the second step. This system has many advantages for the treatment of sludges or wet solids including the fact that it is totally enclosed thereby eliminating fugitive emissions.

4. Simplified Incinerator Technology for Pollution Abatement (SITPA #II) System - consists of an unlined rotary kiln, a combustion chamber heated by oil burners and a cyclone particulate collector, bag house filter and wet scrubber. While this system is simple and low in cost, it is basically a dry feed system, making it more hazardous for disposal of explosives.

Biodegradation has also been considered as a means of disposing of munitions wastes including those encountered in the TNT manufacturing process. The microbial transformation of TNT has been extensively studied with conflicting reports concerning the degradation of the aromatic ring (Pal and Ryon, 1986).

The toxicity of the ring compounds that might form during this process is largely unknown but similar compounds are known to be toxic (Castorina, 1980). Further research has been contracted to Atlantic Research Corporation to study the effectiveness of composting TNT waste. (Renard, 1984 as cited in Pal and Ryon, 1986)

The primary physical method for the breakdown of TNT involves photolysis. Exposure to sunlight or any other source of UV light results in a rapid breakdown of TNT and its by-products, including 2-4- and 2,6-DNT, to a pink residual. Ring cleavage to CO_2 and volatile organics has been shown (Andrews and Osmon, 1976 as cited in Ryon et al., 1984). Treatment of TNT and RDX in an aqueous solution by exposure of a 1000 gal/day pilot test sample to UV light in the presence of ozone resulted in a reduction of dissolved TNT and RDX to <1 mg/L. By converting the TNT rapidly to CO_2, HNO_3 and H_2O, no by-products requiring disposal are formed (Roth and Murphy, 1978 as cited in Castorina, 1980). Further study of this process awaits the development of more cost-effective equipment.

Several bench-scale models have undergone testing at the Holston AAP Industrial Liquid Waste Treatment Facility for their ability to degrade TNT, alone or in combination with other munition chemicals, in aqueous solutions. These models include:

1. Corona Oxidation (Innova Process) - uses electrolysis and graphite fiber particles; results in degradation, both singly and in mixtures; degradation products not identified; both oxidation and reduction processes seem to occur (Kobylinski and Burrows, 1983).

2. Combined UV Radiation-Ozone Treatment - apparently useful for removal of TNT from relatively small and clean process streams (Burrows, 1983).

3. UV Radiation with Hydrogen Peroxide - results in an increased rate of destruction of TNT when 0.01% hydrogen peroxide is added to mixed munition wastes (Noss and Chyrak, 1984).

Pilot scale testing of authentic wastewaters would be the next step in evaluating those models considered most promising as tertiary treatment methods.

XI. CONCLUSIONS AND RECOMMENDATIONS

Based on the available animal toxicity and human epidemiological data, the HA for One-day, Ten-days and Longer-term exposures is 20 ug/L. The DWEL for TNT is 20 ug/L for lifetime exposure and the Lifetime HA, assuming 20% relative source contribution, is 2 ug/L. TNT is classified EPA Group C, possible human carcinogen. The classification of TNT in EPA Group C is based upon limited animal data. A quantitative cancer risk assessment, based on the limited data, is provided to support selection of uncertainty factors for the recommended lifetime HA.

As indicated in the companion report, "Data Deficiencies/Problem Areas and Recommendations for Additional Data Base Development for Trinitrotoluene" (Appendix 3), standard reproductive and developmental toxicity studies were not available and should be considered in future medical research plans. Also, ophthalmological evaluation of workers in occupational settings should be routinely performed.

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APPENDIX 1

Incidence of Tumors in Animals
Fed TNT in the Diet for 2 Years

Table A1-1. Incidence of Urinary Bladder Lesions in Female Rats Fed TNT for up to 24 months[a/]

	Dose (mg/kg/day)				
Lesion	0.0	0.4	2.0	10.0	50.0
Mucosal Hyperplasia	1/54	0/54	0/55	2/55	12/55[c/]
Transitional cell Papilloma	0/54	0/54	0/55	1/55	5/55[b/]
Transitional-squamous Cell Carcinomas	0/54	0/54	0/55	0/55	12/55[c/]
Combined Papilloma Carcinoma	0/54	0/54	0/55	1/55	17/55

Reference: Furedi et al. (1984a,c)

a/ Number with lesion/number necropsied. Includes those rats sacrificed at the end of the study along with spontaneous deaths and moribund sacrifices (12-24 months)

b/ $p<0.05$

c/ $p<0.01$

APPENDIX 2

Incidence of Myelofibrosis in Rats
Fed TNT in the Diet for 2 Years

Table A2-1. Incidence of Bone Marrow Myelofibrosis in Female Rats Fed TNT for up to 24 months[a]

	Dose (mg/kg/day)				
Lesion	0.0	0.4	2.0	10.0	50.0
Myelofibrosis of Sternal Bone Marrow	5/54	6/53	13/55[b]	12/54	17/54[c]

References: Furedi et al. (1984 a,c)

a/ Number with lesion/number necropsied. Includes those rats sacrificed at the end of the study along with spontaneous deaths and moribund sacrifices (12-24 months)

b/ $p<0.05$

c/ $p<0.01$

APPENDIX 3

Data Deficiencies/Problem Areas and Recommendations For Additional Data Base Development For Trinitrotoluene

INTRODUCTION

The Office of Drinking Water (ODW), Environmental Protection Agency (EPA), in conjunction with the Department of the Army, has reviewed the available data on 2,4,6-trinitrotoluene (TNT) for the purpose of developing a Health Advisory (HA) useful in dealing with contamination of drinking water, to include "state-of-the-art" information on health effects, analytical methodology and treatment technology.

OBJECTIVES

The objective of this appendix is to provide an evaluation of data deficiencies and/or problem areas encountered in the review process for TNT and to make recommendations, as appropriate, for additional data base development. This document is presented as an independent analysis of the current status of TNT toxicology, as relates to its possible presence in drinking water, and includes a summary of the background information used in the development of the HA. For greater detail on the toxicology of TNT, the Health Advisory on Trinitrotoluene should be consulted.

BACKGROUND

Trinitrotoluene, a pale yellow to white crystalline substance, is the most widely used military high-explosive with applications in shells, bombs, grenades, demolition explosives and propellant compositions (Department of the Army, 1967). It is produced at certain selected Army Ammunition Plants (AAP's) and loaded at other AAP's. Production in the United States between 1969-1971 was approximately 45 million pounds per month with a total capacity of 85 million pounds per month (Patterson et al., 1976 as cited in Ryon et al., 1984)

It has been reported that as much as one half million gallons of wastewater have been generated per day at a single plant involved in TNT production (Hartley et al., 1981). The pollutants of greatest concern are in wastewater discharges from manufacturing, purification and load and pack (LAP) processes at the AAPs, and are primarily designated "red water" and "pink water" (Ryon et al., 1984). These two major types of wastes differ substantially both in composition and treatment techniques.

Red water arises from the sellite purification process for crude TNT, is high in solids and, in one recovery study, contained approximately 0.6% TNT by weight as well as other substances, largely as suspended solids (Ryon et al., 1984). Pink water results from plant clean-up and scrubbers processes, LAP operations and red water treatment procedures and contains, in addition to approximately 1% 2,4,6-TNT, the other TNT isomers and numerous degradation products (Spanggord et al., 1978). Continuous flow raw wastewater from one AAP, prior to its final treatment by neutralization and sedimentation, was estimated to contain an average of 20 mg/L of α-TNT (Nay et al., 1974 as cited in Ryon et al., 1984). These values varied from <0.05 to 210 mg/L depending on the site of sampling, production levels and stage of production and/or treatment (Spanggord et al., 1978).

Treatment technology for red and pink waters differs considerably. While the volume of pink water generated is considerably higher, the handling of red waters poses the greatest health risk. A sulfite recovery process (SONOCO Process; Ryon et al., 1984) appears most promising for treatment of red water via recovery of sellite and conversion of impurities to natural products. Current treatment of pink water involves adsorption on activated carbon. A process to regenerate the spent carbon, if successful, could increase the efficiency and efficacy of this process.

Various laboratory studies (Jerger et al., 1976; Spanggord et al., 1980) indicate that the persistence of TNT in the aquatic environment, while varying somewhat due to physical and biological conditions, is considered low due to degradation both by photolysis in the water column and bacterial species in sediments. Persistence in soil and groundwater is lengthier with one study (Sanocki et al., 1976 as cited in Ryon et al., 1984) indicating a level over 3000 mg/kg in a former waste lagoon now filled with sediment and coal wastes, 20 years after its active use as a disposal site. Bioaccumulation in organisms is not considered a significant problem (Ryon et al., 1984).

The pharmacokinetic properties of TNT have been studied in dogs, rats, mice and rabbits. Available data indicate that it is well absorbed by inhalation, ingestion or skin contact, detoxified by the liver with a low distribution to other tissues, and is excreted primarily in the urine. It is metabolized largely by reduction of the nitro group, extensively in all four species and similarly in rats, mice and dogs (Hodgson et al., 1977).

Metabolites include the hydroxylamines, the monoaminodinitro and diaminomononitro derivatives. Little unchanged TNT was found. Quantitative differences in metabolites were evident when different treatment routes were used. Rabbits appeared to metabolize TNT at least quantitatively different than other species. A red pigment in the urine, believed to result from a metabolite of TNT and evident in humans exposed to TNT in the workplace, was also found in rats and mice but its source could not be identified. (El-hawari et al., 1981).

While numerous effects of TNT in humans exposed during the manufacturing process have been reported, the most persistent effects involve the hematopoietic system and the liver (Zakhari and Villaume, 1978). Occupational health surveys (Friedlander et al., 1974; Morton and Ranadive, 1974; Buck and Wilson, 1975) indicate that these effects are generally detectable by changes in the hematological and chemical parameters of the blood and are readily reversible upon removal of the individual from the source of exposure.

Cataract development in occupationally exposed workers has been reported (Hassman and Juran, 1968; Hassman et al., 1978; Harkonen et al., 1983; Makitie et al., 1984), but not among U.S. munitions workers.

Acute toxicity studies were conducted in rats and mice with LD_{50}s ranging from approximately 800 to 1320 mg/kg in male and female rats and from 660 to 1015 mg/kg in male and female mice (Lee et al., 1975; Dilley et al., 1978, 1982). Toxic signs included inactivity with tremors, proceeding to symmetrical coordinated convulsions, with death usually due to respiratory paralysis. Survivors showed signs of ataxia and cyanosis. Red urine was noted in both species within 10-60 minutes of dosing.

In skin and eye irritation tests, TNT produced no to mild irritation of rabbit skin but did not irritate the eye when washed within five minutes. Longer periods of contact resulted in iritis and corneal opacity. In the guinea pig, TNT was a moderate sensitizing agent (Lee et al., 1975; Newell et al., 1976). Red staining of the skin and tissues surrounding the eye was evident.

Four-week feeding studies conducted by Levine et al. (1984a as Appendix IV in Furedi et al., 1984d) in B6C3F1 mice at levels up to 700 mg/kg/day produced no mortality and few toxic signs. At the high dose, body weight was low, bilirubin was increased, kidneys and testes of males were significantly decreased in weight and white blood cell counts were low. Females had increased platelet levels. The only significant pathological lesion found in this study was a diffuse increase in the amount of hemosiderin-like pigment in the red pulp of the spleen. At 100 mg/kg/day, body weights were only occasionally and slightly decreased, bilirubin was increased and hemosiderosis of the spleen was of minimal severity. Red urine was evident at high dose levels.

Dilley et al. (1978, 1982) conducted four-week feeding studies in dogs, rats and mice at levels up to 20, 100 and 185 mg/kg/day, respectively. Red urine appeared in dogs at the high dose and in rats and mice at the two highest levels. Significant findings in all three species included a decrease in body weight with an accompanying decrease in food intake, mild to moderate anemia, and increased spleen weights with hemosiderosis. Rats also showed signs of testicular atrophy and increased liver weights while dogs and rats had increased cholesterol levels and decreased serum glutamic pyruvic-acid transaminase (SGPT) activity. In the one dog/sex/level allowed to recover for four weeks, iron levels were greatly increased in both sexes. In the rat study signs of testicular atrophy and hemosiderosis of the spleen were also seen at the next highest treatment level, approximately 42 mg/kg/day. Effects of TNT on mice were minimal.

This four-week feeding study was continued in surviving animals for an additional nine weeks (Dilley et al., 1978, 1982). Rats showed a persistent negative effect on body weight while anemia remained evident in all three species. Rats and mice had hemosiderosis of the spleen and dogs and mice had elevated liver weight with some necrosis evident in mice. Cholesterol levels were increased and SGPT activity was decreased in dogs and rats but were not studied in mice. Only one death occurred in these studies with one dog dying during Week 12. Signs of anemia were minimal in dogs. In the rats, anemia and hemosiderosis of the spleen were also evident at the 35/mg/kg/day level. Iron levels were decreased in males at all but the lowest dose and glucose was decreased in the two lowest dose levels. High-dosed male rats fed TNT for 13 weeks were reported to have testicular atrophy and hyperplasia of the interstitial cells. The only effect reported as treatment-related for mice fed TNT for 13 weeks was hemosiderosis of the spleen. Some necrosis of the liver was seen in the mice allowed to recover for an additional four weeks.

A 13-week feeding study conducted by Levine et al. (1981, 1984b) in Fischer 344 rats fed TNT at levels up to 300 mg/kg/day produced similar results. Lethargy and ataxia were observed early in the study. Small testicular size was noted by palpation during Week 6 in the high-dosed males. Reductions in body weight were seen at most dose levels in males and in females at 125 and 300 mg/kg/day. Other dose-related findings included anemia, elevated serum cholesterol levels, hepatomegaly, testicular atrophy, and increased spleen and kidney weights. Histopathology revealed brain lesions, hepatocytomegaly, increased pigmentation of the spleen and the kidney, splenic congestion and testicular lesions.

In a 90-day feeding study in monkeys (Martin, 1974) and dogs (Hart, 1974), the only effects reported as possibly treatment-related were necrotic or abnormal magakaryocytes in high-dosed female monkeys (1.0 mg/kg/day) with increased iron-positive material in the liver cord cytoplasm and a slight increase in hemosiderosis of the bone marrow in high-dosed (1.0 mg/kg/day) dogs.

Levine et al. (1983) also studied the effects of TNT on dogs dosed over a 26 week period. Two deaths occurred at the highest dose level (32 mg/kg/day). Other effects at this level included dehydration, weight loss, jaundice, hypothermia, diarrhea, ataxia, anemia, elevations in bilirubin, serum globulin and lactic dehydrogenase (LDH), and decreases in SGPT and glucose. Histological examination revealed hemosiderosis of the spleen and liver, splenic congestion, increased spleen and liver weights, hepatocytomegaly with hepatocytic cloudy swelling and cirrhosis. Effects on the liver were evident at all dose levels (0.5 to 32 mg/kg/day), although minimally so at the lowest doses.

Lifetime feeding studies were conducted by Furedi et al. (1984a-f) in Fischer 344 rats and B6C3F1 mice at levels up to 50 and 70 mg/kg/day, respectively. Significant adverse effects in rats fed TNT for two years included decreases in weight gain and food consumption, anemia, methemoglobinemia, myelofibrosis of the bone marrow, congestion, extramedullary hematopoiesis and hemosiderosis of the spleen, increases in liver, kidney and spleen weights, hepatocellular hyperplasia with cystic degeneration, alterations in lipid and protein metabolism, spotted and cystic kidneys with pigmentation, inflammation and lymphocytic infiltration, hyperplasia of the renal pelvis and urinary bladder hyperplasia, papilloma and carcinoma. Several effects were significant at the 2 mg/kg/day dose level.

In the study in mice, effects related to TNT intake included mild decreases in weight gain, anemia, occasional elevations in relative weights of the liver, kidneys, spleen and heart, extramedullary hematopoiesis of the spleen, cytoplasmic vacuolization of the renal tubules (males), renal lymphocytosis and enlarged spleen and lymph nodes (females). Effects in mice were seen in animals receiving 10 mg/kg/day or more.

Trinitrotoluene was reported to be strongly mutagenic in Salmonella typhimurium in all test strains at various dose levels (Ellis et al., 1978). In vivo cytogenic analysis on bone marrow cells from rats revealed no evidence of genetic damage induced by TNT while an in vitro measurement of unscheduled DNA synthesis (UDS) in human diploid fibroblasts suggested a positive response at higher dose levels (Dilley et al., 1978).

Ashby et al. (1985) reported a negative response in a mouse bone marrow micronucleus assay while an in vivo/in vitro rat liver assay for UDS was also negative.

Intake of TNT in the diet for two years at levels up to 50 mg/kg/day in rats was reported to cause cancer in the female animals. Female rats fed TNT at 10 and 50 mg/kg/day showed an increase in the incidence and severity of hyperplastic, preneoplastic and neoplastic lesions of the mucosal epithelium of the urinary bladder (Furedi et al., 1984a-f).

No studies on the possible reproductive or developmental effects of TNT were found. However, potential reproductive effects were noted in 13-week feeding studies (Levine et al., 1981, 1984b).

Several methods of chemical analysis for TNT in water have been reported with a high performance liquid chromatography (HPLC) method apparently suitable for detection of TNT in LAP discharge wastewater samples. The lower limit of detection was reported to be 0.1 ppm (Spanggord et al., 1978).

Treatment of "red water" and "pink water" wastes is accomplished differently. A sulfite recovery (SONOCO) process appears most feasible for treating red water waste and has been adapted for use at the Radford AAP (Ryon et al., 1984).

Granular activated carbon (GAC) adsorption is the current method for treating pink water wastes. A method to regenerate the spent carbon is under development (Pal and Ryon, 1986) but the presence of other munitions in the wastewater places limitations on the GAC method (Burrows, 1982). An alternate hydroperm microfiltration system is under development (Sundaram et al., 1981 as cited in Pal and Ryon, 1986).

Based on the significant findings of the foregoing studies, HA values for One-day, Ten-days and Longer-Term were established at 20 µg/L, the Drinking Water Equivalent Level (DWEL). A DWEL is defined as the medium-specific (in this case, drinking water) exposure which is interpreted to be protective for non-carcinogenic endpoints of toxicity over a lifetime of exposure. This DWEL is calculated for a 70 kg adult consuming 2 liters of water per day. The Lifetime HA is 2 ug/L and assumes 20% relative source contribution. TNT is classified as EPA Group C, possible human carcinogen.

The estimated excess cancer risks associated with lifetime exposure to drinking water containing TNT at 20 µg/L and 2 µg/L have been calculated to be 2×10^{-5} and 2×10^{-6}, respectively.

DISCUSSION

Available data on the pharmacokinetics, health effects, analysis and treatment of TNT have been reviewed.

The pharmacokinetic properties of TNT have been studied in various species and results indicate that it is easily absorbed, and that metabolism is qualitatively similar, if quantitatively different, in all three species. Little data is available on the metabolism in humans; however, humans as well as rats, mice and dogs (at high-dose levels) produce a metabolite, as yet unidentified, that causes a red color to appear in the urine. Identification of this color-producing metabolite could be significant to the metabolic profile of TNT but does not necessarily impact on the development of a HA.

The available studies on the toxicity of TNT include LD_{50}s in rats and mice, short-term (four-weeks in dogs, rats and mice) and longer-term (13-weeks in dogs, rats, mice and monkeys; 26-weeks in dogs; and 24-months in rats and mice) studies including assessments for carcinogenic potential. Results of these studies produced similar results in most species with effects on the hematopoietic system (anemia with related effects in the spleen) apparent from the shortest term through lifetime studies. Effects on the hepatic and renal systems became apparent with increasing length of exposure. Available data following human exposure to TNT in the workplace indicate similar effects in the various human systems. The only seeming inconsistency in these studies was the testicular atrophy apparent in the several studies in rats fed TNT for up to 13-weeks but not evident in a lifetime study in this species. Data suitable for One-day, Ten-day and Longer-term HAs are unavailable but the

DWEL of 20 µg/L is considered to be a conservative estimate for safe exposure levels for non-carcinogenic endpoints of toxicity over a lifetime of exposure. These data are supported by consistent effects in the various longer-term studies.

In view of the testicular effects in rats, the need for a three-generation reproduction study along with developmental studies, both of which are currently lacking, is of primary importance.

While the various mutagenic assays differed somewhat in their results, most negative data occurred at low doses with positive results for mutagenic activity predominating at the higher doses. The results of *in vivo* and *in vitro* assays for cytogenetic effects were somewhat uncertain, largely due to limitations based on solubility of TNT in the various systems and to interference from discolorations in the samples. Nevertheless, mutagenicity studies are adequate.

Evidence has been presented that TNT is carcinogenic to rats. The carcinomas of the bladder in female rats fed TNT for 2 years were not previously indicated by short-term toxicities (but identification of the red color-producing metabolite of TNT becomes of possible significance). In the mouse study, TNT was administered in the diet for up to 24 months. Groups of 75 mice per sex received TNT at doses of 0, 1.5, 10, or 70 mg/kkg/day. Ten mice per sex per dose were killed following 6 and 12 months on test with surviving animals killed after 24 months of treatment. The major systemic effects observed in the high (70 mg/kg/day) dose group included anemia with hepatotoxicity. This indicates the MTD was achieved. The study authors reported that the incidence of combined leukemia/malignant lymphoma in the spleen of females increased with dose. They reported that the increase was statistically significant ($p<0.05$) at the 70 mg/kg/day dose level (high dose) and that the lesions were considered to be treatment-related. This was an inappropriate conclusion based upon current NTP guidelines (McConnell et al., 1986). These guidelines indicate that it is appropriate to combine all types of malignant lymphoma and lymphocytic leukemia, but not in a single organ. These types of tumors occur throughout the hematopoietic system. Upon recounting these tumors, by each sex or both sexes combined, in the whole animal, the statistical significance is lost (i.e., $p>0.05$) using the Fisher-Irwin Exact Test to compare dosage groups and the Cochran-Armitage Test for Trend. Therefore, based upon the statistical analyses, this study is considered to be negative with no tumors related to TNT exposure.

It has been reported in the literature that TNT may be associated with cataract formation in humans (Hassman and Juran, 1968; Hassman et al., 1978; Zakhari and Villaume, 1978; Harkonen et al., 1983; Makitie et al., 1984). Similar findings have not been reported among munitions workers in the United

States nor has this finding been supported by data in rats and mice subjected to extensive ophthalmic examinations at various periods throughout the 2-year feeding studies.

Several methods of analysis for TNT in wastewater have been reported. One method utilizing HPLC appears to be capable of being adapted to a sensitivity level suitable for detecting those levels of TNT that may be considered hazardous to health. The lower limits of detection for this method were reported as 0.1 ppm, lowerable by a factor of 2 at 210 nm (equivalent to 50 μg/L for this lower limit of detectability (Spanggord et al., 1978)).

Methods for treating both "red" and "pink" waters are available. Since red water has been declared a "hazardous waste" by the EPA (Ryon et al., 1984), its disposal by concentration, incineration and landfilling of the ash is no longer considered suitable. Alternate methods of treatment are under active investigation (Ryon et al., 1984) and an adaptation of a sulfite recovery process (SONOCO) currently being used at the Radford AAP appears to be a safe and effective treatment method for this toxic waste.

Treatment of pink water, while not presenting as hazardous a waste disposal problem, does present problems of economic feasibility due to the quantities of pink water wastes generated during the various production processes. Adsorption by activated carbon has been and remains a viable treatment method. Methods to regenerate the spent carbon are undergoing active investigaton (Forstan, 1980 as cited in Ryon et al., 1984) and their development should relieve some of the economic burden of this waste treatment method. An hydroperm microfiltration system is also undergoing active study and may provide an alternate treatment method (Sundaram et al., 1981 as cited in Pal and Ryon, 1986). Both analysis and treatment methods for TNT in wastewaters are adequate.

CONCLUSIONS/RECOMMENDATIONS

Based on the above discussion, the following conclusions/recommendations are made:

1. The available studies on the toxicity of TNT are generally considered adequate for development of a HA useful in dealing with the potential contamination of drinking water.

2. No data are available on the reproductive and developmental effects of TNT. In view of the testicular effects seen in rats fed TNT for periods up to 13-weeks, a three-generation reproduction study and a study to determine possible developmental effects, utilizing currently accepted protocols, is recommended.

3. Available data on the occurence of cataracts in European workers clearly indicate that TNT does produce this effect. However, actual air levels, absorbed dose, and mechanisms of toxicity are not clear. These difficulties are compounded by the fact that cataracts have not been noted in U.S. TNT-workers. Cataract development is an effect that must be clarified by further studies to determine the dose and mechanism producing this effect. Additional studies in individuals exposed to TNT in occupational settings should be considered. In particular, thorough ophthalmological examinations should be routinely performed.

4. Aside from the aforementioned data gaps, no further studies on TNT, as relates to its possible presence in drinking water, are deemed necessary at this time.

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